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August 17, 2000

ATTN: BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

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Re: Application of **Mitsuyuki MATSUMOTO, Toru SUGIMOTO, Jun TAKASAKI, Masazumi KAMOHARA, Tetsu SAITO and Masato KOBAYASHI**
NOVEL G PROTEIN-COUPLED RECEPTOR PROTEINS
Our Reference: Q60438
PCT/JP99/01191, filed March 11, 1999

Dear Sir:

Applicants herewith submit the attached papers for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter II of the Patent Cooperation Treaty. Attached hereto is the application identified above which is a translation **of PCT International Application No. PCT/JP99/01191, filed March 11, 1999**, comprising the specification, claims, ten (10) sheets of drawings, executed Declaration and Power of Attorney, Sequence Listing, a 3.5" disk containing Sequence Listing in a computer readable form, Statement in Support of Submission in Accordance with 37 C.F.R. § 1.821, Notification Concerning Submission or Transmittal of Priority Document, International Preliminary Examination Report (foreign language), Preliminary Amendment, Amended Abstract, International Search Report, Information Disclosure Statement, PTO Form 1449 with reference, executed Assignment and PTO Form 1595.

The Government filing fee is calculated as follows:

Total Claims	12 - 20 =	0 x $18 =	$ 000.00
Independent Claims	2 - 3 =	0 x $78 =	$ 000.00
Base Filing Fee	($840.00)		$ 840.00
Multiple Dep. Claim Fee	($260.00)		$ 260.00
TOTAL FILING FEE			**$1,100.00**
Recordation of Assignment Fee			$ 40.00
TOTAL U.S. GOVERNMENT FEE			**$1,140.00**

Checks for the statutory filing fee of **$ 1,100.00** and Assignment recordation fee of **$ 40.00** are attached. You are also directed and authorized to charge or credit any difference or overpayment to Deposit Account No. 19-4880. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. 1.492; 1.16 and 1.17 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Priority is claimed from:

Japanese Patent Application	Filing Date
P.Hei. 10-060245	March 12, 1998
P.Hei. 11-026774	February 3, 1999

Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of Q60438

Mitsuyuki MATSUMOTO, Toru SUGIMOTO, Jun TAKASAKI, Masazumi KAMOHARA, Tetsu SAITO and Masato KOBAYASHI

Serial No.: NOT YET ASSIGNED PCT/JP99/01191, filed March 11, 1999

Filed: August 17, 2000

For: NOVEL G PROTEIN-COUPLED RECEPTOR PROTEINS

PRELIMINARY AMENDMENT

ATTN: BOX PCT
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Prior to examination of the above-identified application, please amend the above-mentioned application as follows:

IN THE SPECIFICATION:

Page 30, line 7, please delete new paragraph break;

line 11, after "concentrations." please insert a new paragraph break.

REMARKS

The above amendment(s) are made for editorial purposes.

Applicants submit no questions of new matter should arise and entry is requested.

Respectfully submitted,

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Description

Novel G Protein-coupled Receptor Proteins

Technical Field

This invention belongs to the genetic engineering field, and relates to novel G protein-coupled receptor proteins, genes coding for these G protein-coupled receptor proteins, methods for producing these G protein-coupled receptor proteins, screening methods using these G protein-coupled receptor proteins, antibodies for these G protein-coupled receptor proteins and screening methods using these antibodies.

Background Art

Cell membrane receptors which transmit signals to the intracellular region via the activation of heterotrimeric GTP binding protein are generally referred to as "G protein-coupled receptor". All members of the G protein-coupled receptor known to date are sometimes referred generally to as "seven transmembrane receptor", because they form a super family having a common structure which has the extracellular amino terminus and intracellular carboxyl terminus and passes through the cell membrane seven times. The G protein-coupled receptor transmits information on various physiologically active substances

from cell membranes to the intracellular region via activation of heterotrimeric GTP binding protein and subsequent changes in the intracellular second messengers induced. As the intracellular second messengers which are controlled by the heterotrimeric GTP binding protein, cAMP via adenylate cyclase, Ca^{++} via phospholipase C and the like are well known, and it has been revealed recently that many cellular proteins are their targets, such as the control of channels and activation of protein kinases via the heterotrimeric GTP binding protein (Gudermann, T. *et al.* (1997), *Annu. Rev. Neurosci.*, 20, 399 - 427). The physiologically active substances that transmit information via the G protein-coupled receptor include various known physiologically active substances such as neurotransmitters, hormones, chemokine, lipid-originated signal transducers, divalent ions and proteases. Information by these physiologically active substances is transmitted to the intracellular region through their specific G protein-coupled receptor, respectively.

Several hundred types of G protein-coupled receptor have so far been cloned from eucaryote. Regarding human, hundred or more types of G protein-coupled receptor for corresponding endogenous ligands have been cloned and are regarded as targets of drugs for diseases. There are various diseases in which G protein-coupled receptor is the

target, and there exist effective drugs which act upon G protein-coupled receptor, in the respective fields of central nervous system, circulatory organ system, inflammatory immune system, digestive organ system, motor organ system and urinary organ/reproductive organ system (Stadel, J. *et al.* (1997), *Trends Pharmacol. Sci.*, 18, 430 - 437). This indicates that agonists or antagonists of G protein-coupled receptor have a high possibility of becoming a therapeutic agent of diseases, so that studies are being actively carried out on the discovery and identification of new G protein-coupled receptors.

Cloning of G protein-coupled receptor genes tends to start based on their structural homology in the super family in many cases, and a receptor having no correspondence to endogenous ligand is referred to as "the orphan G protein-coupled receptor". In general, a ligand specific for the orphan G protein-coupled receptor has not been found, so that it was difficult to develop its agonist or antagonist. In recent years, however, it has been proposed to create a drug targeting for the orphan G protein-coupled receptor by combining the substantiated compound libraries and high performance high throughout screening (Stadel, J. *et al.* (1997), *Trends Pharmacol. Sci.*, 18, 430 - 437).

That is, it is possible to screen an agonist for an orphan G protein-coupled receptor from a compound library by effective high throughput system of the measurement of cAMP and Ca^{++} which are second messengers of many G protein-coupled receptors, or the measurement of GTPase activity and G protein binding of GTPγS which are indexes of the activation of heterotrimeric GTP binding protein, so that it is possible to find specific agonists and antagonists making use of such compounds and furthermore to develop therapeutic drugs for certain diseases. Under such conditions, discovery of a novel G protein-coupled receptor capable of becoming a new therapeutic target of diseases is regarded as the most important step in creating a medicament which acts upon G protein-coupled receptors.

Among G protein-coupled receptors, there is a case in which a plurality of receptors are present for one endogenous ligand. Such receptors are referred to as receptor family, and each receptor is called subtype. Since all of the G protein-coupled receptors have a common structure which passes through the cell membrane seven times, 20 to 25% of amino acids are preserved mainly in the transmembrane region even in mutually independent G protein-coupled receptors, but when they form a receptor family, ratio of the amino acids preserved among its subtypes significantly increases to 35% or more,

particularly to 60 to 80% among subtypes having high relevancy (Strader, C.D. *et al.* (1994), *Annu. Rev. Biochem.*, 63, 101 - 132).

When development of a therapeutic drug for diseases is planned by targeting for an endogenous ligand wherein a receptor family is present, specificity of its subtypes becomes important in many cases. This is because actions upon other subtype than actions upon a subtype that mediates the main action of a drug lead to side effects in many cases. Accordingly, it is desirable to create a subtype-specific agonist or antagonist, but it is necessary to find a means for detecting the subtype-specificity for that purpose. Currently, a method for constructing a system in which a gene of a subtype is cloned and its specificity is detected using a cultured cell line or the like which expresses the gene is generally used.

When a novel G protein-coupled receptor is used as the target of disease treatment, it is highly possible that the subtype-specificity is important, so that discovery of a receptor family is important also in the case of the novel G protein-coupled receptor. The homology of amino acid sequences among independent G protein-coupled receptors is 20 to 25% as a whole, but when they form a receptor family, the homology significantly increases in general in the family, so that it is possible to presume

whether they form a family or not, by comparing homology between two G protein-coupled receptors. It is possible to find novel G protein-coupled receptors which form a family, making use of such a means, and when a novel G protein-coupled receptor family is discovered, it will open a way for developing a drug for disease therapy because of the possibility of creating a subtype-specific agonist or antagonist.

The central nervous system transmits and controls various kinds of information using physiologically active substances represented by neurotransmitters. The G protein-coupled receptor is taking an important role in the signal transduction and control. Since many types of G protein-coupled receptor are present in the central nervous system, they are used as important therapeutic targets for diseases of the central nervous system. For example, it is considered that the G protein-coupled receptor of a neurotransmitter, dopamine, is a therapeutic target of schizophrenia (Seeman, P. *et al.* (1997), *Neuropsychopharmacology*, 16, 93 - 110), the G protein-coupled receptor of serotonin is that of depression (Cowen, P.J. (1991), *Br. J. Psychiatry*, 159 (Suppl. 12), 7 - 14), and the G protein-coupled receptor of neuro-peptide Y is that of eating disorder (Blomqvist, A.G. and Herzog, H. (1997), *Trends Neurosci.*, 20, 294 - 298).

It is considered that a novel G protein-coupled receptor expressing in the central nervous system, preferably a human receptor, will lead to a candidate for a new therapeutic target of central nervous system diseases or to the elucidation of central nervous system functions. In addition, for the purpose of developing a subtype-specific drug, it is desirable also to find a family in the case of the novel G protein-coupled receptor expressing in the central nervous system. Though the gene of a receptor GPR27 obtained from a mouse, having high homology with the amino acid sequence of SREB1 which is one of the G protein-coupled receptors of the invention, and an amino acid sequence based on its gene sequence have been reported (O'Dowd, B.F. *et al.* (1998), *Genomics*, 47, 310 - 313), no information is available to date concerning gene sequence and amino acid sequence of a human receptor.

Disclosure of the Invention

The present invention is to provide novel G protein-coupled receptor family proteins expressed in the central nervous system, as the target of therapeutic agents for central nervous system diseases.

With the aim of achieving the above object, the present inventors have conducted intensive studies and, as a result, succeeded in isolating genes (SREB1, SREB2,

SREB3, rSREB1, rSREB2 and rSREB3) which encode novel G protein-coupled receptor family proteins expressed in the central nervous system.

Also, we have established vectors containing these genes, host cells containing these vectors and methods for producing these G protein-coupled receptor proteins using such host cells, and rendered possible screening of these G protein-coupled receptor proteins and compounds, peptides and antibodies capable of modifying activities of the G protein-coupled receptor proteins.

Illustratively, the present invention relates to

(1) a G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26, or a G protein-coupled receptor protein as an equivalent to the protein,

preferably a human origin G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 2, 4 or 6 or a G protein-coupled receptor protein as an equivalent to the protein, or a rat origin G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 6, 22 or 26 or a G protein-coupled receptor protein as an equivalent to the protein,

(2) a G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26,

(3) a gene which has a nucleotide sequence coding for the G protein-coupled receptor protein described in the item (1),

(4) a vector which contains the gene described in the item (3),

(5) a host cell which contains the vector described in the item (4),

(6) a method for producing the G protein-coupled receptor protein described in the item (1) or (2), or a G protein-coupled receptor protein as an equivalent to the protein, which comprises using the host cell described in the item (5),

(7) a method for screening a medicament acting on the G protein-coupled receptor protein described in the item (1) or (2), which comprises allowing the G protein-coupled receptor protein to contact with a compound to be tested, or

(8) an antibody for the G protein-coupled receptor protein described in the item (1) or (2) or a partial peptide thereof.

The following explains the terms to be used herein.

The term "human origin" or "rat origin" means an amino acid sequence identical to the amino acid sequence of a G protein-coupled receptor protein expressing in human or rat.

The term "equivalent" of the G protein-coupled receptor protein of the present invention means a G protein-coupled receptor protein which is expressed in the central nervous system and shows the same activity of any one of the G protein-coupled receptor proteins represented by the amino acid sequences described in Sequence No. 2, 4, 6, 22 or 26.

In this connection, the G protein-coupled receptor and the G protein-coupled receptor protein have the same meaning.

The novel G protein-coupled receptor protein of the present invention is any one of the G protein-coupled receptor proteins represented by the amino acid sequences described in Sequence No. 2, 4, 6, 22 and 26, or equivalents thereof. Illustratively, all of G protein-coupled receptor proteins are included in the invention as long as they have the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26, or an amino acid sequence in which the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26, has substitution, deletion or insertion of one or a plurality, preferably from 1 to 10, more

preferably from 1 to 7, most preferably from 1 to 5, of amino acids, and have the same activity of the protein represented by the amino acid sequence described in Sequence No. 2, 4 or 6. Preferably, it is a human or rat origin G protein-coupled receptor protein having the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26.

Also, the gene which has a nucleotide sequence coding for the novel G protein-coupled receptor protein of the invention may be any gene, as long as it has a nucleotide sequence coding for the G protein-coupled receptor protein represented by the amino acid sequence described in Sequence No. 2, 4 or 6, or an equivalent thereof. Preferably, it is a gene which has a nucleotide sequence coding for the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26. More preferably, it is a gene which has a sequence of from 1 to 1,125 positions of the nucleotide sequence described in Sequence No. 1, from 1 to 1,110 positions of the nucleotide sequence described in Sequence No. 3, from 1 to 1,119 positions of the nucleotide sequence described in Sequence No. 5, from 1 to 1,131 positions of the nucleotide sequence described in Sequence No. 21, from 1 to 1,110 positions of the nucleotide sequence described in Sequence No. 23 or from 1 to 1,119 positions of the nucleotide sequence described in Sequence No. 25.

The gene which encodes the G protein-coupled receptor protein of the invention can be obtained by the following methods.

1) Production methods of novel G protein-coupled receptor protein gene

a) First production method

A mRNA sample is extracted from human cells or tissue having the ability to produce the G protein-coupled receptor protein of the invention. Next, using this mRNA as the template, two primers interposing the G protein-coupled receptor protein mRNA or a part of the mRNA region is prepared. The G protein-coupled receptor protein cDNA or a part thereof can be obtained by carrying out a reverse transcriptase-polymerase chain reaction (to be referred to as RT-PCR hereinafter) suited for SREB1, SREB2 or SREB3 by modifying the conditions for denature temperature, denaturing agent addition and the like. Thereafter, the receptor protein can be produced by integrating the thus obtained G protein-coupled receptor cDNA or a part thereof into an appropriate expression vector and expressing it in a host cell.

Firstly, mRNA molecules including those encoding the G protein-coupled receptor protein of the invention are extracted by a known method from cells or tissue, such as of the human brain or rat brain, having the ability to

produce the protein. Regarding the extraction method, a guanidine thiocyanate hot phenol method, a guanidine thiocyanate-guanidine hydrochloride method and the like can be exemplified, and a guanidine thiocyanate cesium chloride method can be cited as a preferred method. The cells or tissue having the ability to produce the protein can be identified by the Northern blotting method using a gene having a nucleotide sequence coding for the protein or a part thereof or by the Western blotting method using an antibody specific for the protein.

Purification of mRNA can be carried out in accordance with the conventional method, for example by adhering the mRNA to an oligo(dT) cellulose column and then eluting it therefrom. In addition, the mRNA can be further fractionated, for example, by a sucrose density gradient centrifugation. Alternatively, a commercially available already-extracted mRNA preparation may be used without carrying out the mRNA extraction.

Next, a single-stranded cDNA is synthesized from the thus purified mRNA by carrying out a reverse transcriptase reaction in the presence of a random primer or an oligo-dT primer. This synthesis can be carried out in the conventional way. The novel G protein-coupled receptor DNA of interest is amplified by subjecting the thus obtained single-stranded cDNA to PCR using two primers interposing a

region of the gene of interest. The thus obtained DNA is fractionated, for example, by an agarose gel electrophoresis. As occasion demands, a DNA fragment of interest can be obtained by digesting the DNA with restriction enzymes and then connecting the digests.

b) Second production method

In addition to the above method, the gene of the invention can also be produced making use of conventional genetic engineering techniques. Firstly, single-stranded cDNA is synthesized using the mRNA obtained by the above method as the template and a reverse transcriptase, and then double-stranded cDNA is synthesized from the single-stranded cDNA. Examples of the method include the S1 nuclease method (Efstratiadis, A. *et al.* (1976), *Cell*, 7, 279 - 288), the Land method (Land, H. *et al.* (1981), *Nucleic Acids Res.*, 9, 2251 - 2266), the O. Joon Yoo method (Yoo, O.J. *et al.* (1983), *Proc. Natl. Acad. Sci. USA*, 79, 1049 - 1053) and the Okayama-Berg method (Okayama, H. and Berg, P. (1982), *Mol. Cell. Biol.*, 2, 161 - 170).

Next, the recombinant plasmid obtained by the above method is introduced into an *Escherichia coli* strain, such as DH5α, to effect its transformation, and a transformant can be selected making use of tetracycline resistance or ampicillin resistance as a marker. For example, when the host cell is *Escherichia coli*, transformation of the host

cell can be carried out by the Hanahan's method (Hanahan, D. (1983), *J. Mol. Biol.*, 166, 557 - 580), namely a method in which the recombinant DNA is added to competent cells prepared in the presence of $CaCl_2$ and $MgCl_2$ or RbCl. In this case, not only a plasmid but also a lambda or the like phage vector can also be used as the vector.

A strain having DNA coding for the novel G protein-coupled receptor protein of interest can be selected from the thus obtained transformants, for example by the following various methods.

(1) A screening method which uses a synthetic oligonucleotide probe

An oligonucleotide corresponding to the entire portion or a part of the G protein-coupled receptor protein of the invention is synthesized (in this case, it may be either a nucleotide sequence derived using the codon usage or a combination of plural possible nucleotide sequences, and in the latter case, their kinds can be reduced by including inosine), this is used as a probe (labeled with ^{32}P or ^{33}P) and allowed to hybridize with DNA samples of transformants, which are denatured and fixed on a nitrocellulose filter, and then a positive strain is screened and selected.

(2) A screening method which uses a probe prepared by polymerase chain reaction

Sense primer and antisense primer oligonucleotides corresponding to a part of the G protein-coupled receptor protein of the invention are synthesized, and polymerase chain reaction (Saiki, R.K. *et al.* (1988), *Science*, 239, 487 - 491) is carried out using a combination of them to effect amplification of a DNA fragment of interest coding for the entire portion or a part of the G protein-coupled receptor protein. As the template DNA to be used herein, cDNA synthesized by the reverse transcription reaction from mRNA of cells capable of producing the G protein-coupled receptor protein or genomic DNA can be used. The thus prepared DNA fragment is labeled with ^{32}P or ^{33}P and used as the probe to select a clone of interest by carrying out colony hybridization or plaque hybridization.

(3) A screening method in which the novel G protein-coupled receptor protein is produced in other animal cells

A transformant is cultured to amplify the gene of interest, the gene is transfected into an animal cell (in this case, either a plasmid which can perform autonomous replication and contains a transcription promoter region or a plasmid which can be integrated into chromosome of the animal cell may be used) and a protein coded by the gene is produced on the cell surface. By detecting the protein

using an antibody specific for the G protein-coupled receptor protein of the invention, a strain of interest having cDNA coding for the G protein-coupled receptor protein is selected from the original transformants.

(4) A selection method which uses an antibody specific for the G protein-coupled receptor protein of the invention

In advance, cDNA is integrated into an expression vector and protein is produced on the surface of transformant strains, and then strains capable of producing the G protein-coupled receptor protein are detected using an antibody specific for the G protein-coupled receptor protein of the invention and a second antibody for the first antibody, thereby selecting a strain of interest.

(5) A method which uses a selective hybridization translation system

Samples of cDNA obtained from transformants are blotted on, for example, a nitrocellulose filter and hybridized with mRNA prepared from cells capable of producing the G protein-coupled receptor protein of the invention, and then the mRNA linked to the cDNA is dissociated and recovered. The thus recovered mRNA is then translated into protein using a protein translation system, for example by injecting into Xenopus oocyte or in a cell-free system such as a rabbit reticulocyte lysate, wheat germ or the like. A strain of interest is selected by

detecting it using an antibody for the G protein-coupled receptor protein of the invention.

Collection of DNA which encodes the G protein-coupled receptor protein of the invention from the thus obtained transformant of interest can be carried out in accordance with a known method (Maniatis, T. *et al.* (1982): "Molecular Cloning - A Laboratory Manual", Cold Spring Harbor Laboratory, NY). For example, it can be carried out by separating a fraction corresponding to a plasmid DNA from cells, and cutting out a cDNA region from the plasmid DNA.

c) Third production method

The gene which has a nucleotide sequence coding for the amino acid sequence represented by Sequence No. 2, 4, 6, 22 or 26 can also be produced by binding DNA fragments produced by a chemical synthesis method. Each DNA can be synthesized using a DNA synthesizer (e.g., Oligo 1000M DNA Synthesizer (Beckman), 394 DNA/RNA Synthesizer (Applied Biosystems) or the like).

d) Fourth production method

For the purpose of effecting expression of the function of G protein-coupled receptor protein of the invention by the substance thus obtained by genetic engineering techniques making use of the gene of the invention, it is not always necessary to have all of the amino acid sequences represented by Sequence No. 2, 4, 6,

22 and 26; for example, even if it is a partial sequence or other amino acid sequence is added thereto, such proteins are also included in the G protein-coupled receptor protein of the invention, as long as they show the same activity of the G protein-coupled receptor protein represented by the amino acid sequence shown in Sequence No. 2, 4, 6, 22 or 26. Also, as is known by the interferon gene and the like, it is considered that genes of eucaryote generally show polymorphism (e.g., see Nishi, T. *et al.* (1985), *J. Biochem.*, 97, 153 - 159), and there is a case in which one or a plurality of amino acid are substituted by this polymorphism or a case in which the nucleotide sequence is changed but the amino acids are completely unchanged. In consequence, even in the case of proteins in which one or a plurality of amino acid residues are substituted, deleted or inserted at one or a plurality of positions in the amino acid sequence represented by Sequence No. 2, 4 or 6, it is possible that they have the same activity of the G protein-coupled receptor represented by the amino acid sequence described in Sequence No. 2, 4 or 6. These proteins are called equivalents to the G protein-coupled receptor protein of the invention and included in the invention. In addition, a G protein-coupled receptor having the rat origin amino acid sequence shown by Sequence No. 22, 24 or

26 or a G protein-coupled receptor having the same activity of the former receptor is also included in the equivalents.

All of the genes having nucleotide sequences which encode these equivalents to the G protein-coupled receptor protein of the invention are included in the invention. Such various genes of the invention can also be produced by nucleic acid chemical synthesis methods in accordance with a usual method such as the phosphite triester method (Hunkapiller, M. *et al.* (1984), *Nature*, 10, 105 - 111), based on the information on the G protein-coupled receptor protein of the invention described in the foregoing. In this connection, codons for desired amino acid are well known, and they can be optionally selected and determined in the usual way, for example by taking codon usage of the host to be used into consideration (Crantham, R. *et al.* (1981), *Nucleic Acids Res.*, 9, r43 - r74). In addition, partial modification of codons of these nucleotide sequences can be carried out in the usual way in accordance, for example, with the site specific mutagenesis (Mark, D.F. *et al.* (1984), *Proc. Natl. Acad. Sci. USA*, 81, 5662 - 5666) which uses a primer comprised of a synthetic oligonucleotide coding for the desired modification.

Determination of the sequence of DNA obtained by the above methods a) to d) can be carried out by, for example, the Maxam-Gilbert chemical modification method (Maxam, A.M.

and Gilbert, E. (1980): "Methods in Enzymology", 65, 499 - 559) or the dideoxy nucleotide chain termination method (Messing, J. and Vieira, J. (1982), *Gene*, 19, 269 - 276) which uses M13.

Also, the vector of the invention, the host cell of the invention and the G protein-coupled receptor protein of the invention can be obtained by the following methods.

2) Production method of recombinant protein of the G protein-coupled receptor of the invention

An isolated fragment containing a gene coding for the G protein-coupled receptor protein of the invention can transform other eucaryotic host cell by again integrating into an appropriate vector DNA. In addition, it is possible to express the gene in respective host cells by introducing an appropriate promoter and a sequence related to the gene expression into these vectors.

Cells of vertebrates, insects, yeast and the like are included in the eucaryotic host cells and, though not particularly limited, examples of commonly used vertebrate cells include COS cell which is a simian cell (Gluzman, Y. (1981), *Cell*, 23, 175 - 182), a dihydrofolate reductase deficient strain of Chinese hamster ovary cell (CHO) (Urlaub, G. and Chasin, L.A. (1980), *Proc. Natl. Acad. Sci. USA*, 77, 4216 - 4220), human fetal kidney HEK293 cell and

293-EBNA cell (Invitrogen) prepared by introducing Epstein Barr virus EBNA-1 gene into the human cell.

As the expression vector for vertebrate cells, a vector which contains a promoter positioned on the upstream of the gene to be expressed, an RNA splicing site, a polyadenylation site, transcription termination sequence and the like can generally be used, and it may further contain a replication origin as occasion demands. Examples of the expression vector include pSV2dhfr having SV40 early promoter (Subramani, S. *et al.* (1981), *Mol. Cell. Biol.*, 1, 854 - 864), pEF-BOS having human elongation factor promoter (Mizushima, S. and Nagata, S. (1990), *Nucleic Acids Res.*, 18, 5322), pCEP4 having cytomegalovirus promoter (Invitrogen) and the like, though not limited thereto.

In a case in which COS cell is used as the host cell, an expression vector which has SV40 replication origin, can perform autonomous growth in COS cell and has a transcription promoter, a transcription termination signal and an RNA splicing site can be used, and its examples include pME18S (Maruyama, K. and Takebe, Y. (1990), *Med. Immunol.*, 20, 27 - 32), pEF-BOS (Mizushima, S. and Nagata, S. (1990), *Nucleic Acids Res.*, 18, 5322), pCDM8 (Seed, B. (1987), *Nature*, 329, 840 - 842) and the like. The expression vector can be incorporated into COS cell by, for example, the DEAE-dextran method (Luthman, H. and

Magnusson, G. (1983), *Nucleic Acids Res.*, 11, 1295 - 1308), the calcium phosphate-DNA co-precipitation method (Graham, F.L. and van der Ed., A.J. (1973), *Virology*, 52, 456 - 457), a method which uses FuGENE6 (Boeringer Mannheim) or the electroporation method (Neumann, E. *et al.* (1982), *EMBO J.*, 1, 841 - 845), and a desired transformant cell can thus be obtained.

Also, when CHO cell is used as the host cell, a transformant cell capable of stably producing the novel G protein-coupled receptor protein can be obtained by carrying out co-transfection of an expression vector together with a vector capable of expressing *neo* gene which functions as a G418 resistance marker, such as pRSVneo (Sambrook, J. *et al.* (1989): "Molecular Cloning - A Laboratory Manual", Cold Spring Harbor Laboratory, NY) or pSV2-neo (Southern, P.J. and Berg, P. (1982), *J. Mol. Appl. Genet.*, 1, 327 - 341), and selecting a G418 resistant colony. In addition, when 293-EBNA cell is used as the host cell, a desired transformant cell can be obtained using an expression vector which has Epstein Barr virus replication origin and can perform autonomous growth in the 293-EBNA cell, such as pCEP4 (Invitrogen).

The thus obtained desired transformant can be cultured in the conventional way, and the G protein-coupled receptor protein of the invention is produced inside the

cells or on the cell surface by this culturing. Regarding the medium to be used in this culturing, it can be optionally selected from various commonly used media depending on each host cell employed; for example, in the case of the COS cell, RPMI-1640 medium, Dulbecco's modified Eagle's minimum essential medium (DMEM) or the like can be used by adding serum components such as fetal bovine serum (FBS) and the like as occasion demands. Also, in the case of the 293-EBNA cell, Dulbecco's modified Eagle's minimum essential medium (DMEM) or the like medium supplemented with serum components such as fetal bovine serum (FBS) and the like can be used by further adding G418.

The G protein-coupled receptor protein of the invention thus produced inside the cell or on the cell surface of the transformant can be separated and purified therefrom by various known separation techniques making use of physical properties, chemical properties and the like of the receptor protein. Illustrative examples of such techniques, to be carried out after solubilization of the receptor protein-containing membrane fraction, include usual treatment with a protein precipitant, ultrafiltration, various liquid chromatography means such as molecular sieve chromatography (gel filtration), adsorption chromatography, ion exchange chromatography, affinity chromatography, high performance liquid

chromatography (HPLC) and the like, dialysis and combinations thereof. In this connection, the membrane fraction can be obtained in the usual way. For example, it can be obtained by culturing the cells which expressed the G protein-coupled receptor protein on the surface, suspending them in a buffer and then homogenizing and centrifuging them. Also, when the G protein-coupled receptor protein is solubilized using a solubilizing agent as mild as possible (CHAPS, Triton X-100, digitonin or the like), characteristics of the receptor can be maintained after the solubilization.

By effecting expression of the G protein-coupled receptor protein of the invention through its in-frame fusion with a marker sequence, confirmation of the expression the G protein-coupled receptor protein, confirmation of its intracellular localization, purification thereof and the like become possible. Examples of the marker sequence include FLAG epitope, Hexa-Histidine tag, Hemagglutinin tag, myc epitope and the like. Also, when a specific sequence recognizable by a protease such as enterokinase, factor Xa or thrombin is inserted between a marker sequence and the G protein-coupled receptor protein, the marker sequence can be cut and removed by such a protease. For example, there is a report in which muscarinic acetylcholine receptor and Hexa-

Histidine tag are connected with a thrombin-recognizing sequence (Hayashi, M.K. and Haga, T. (1996), *J. Biochem.*, 120, 1232 - 1238).

A method for the screening of compounds, peptides and antibodies capable of modifying activity of the G protein-coupled receptor protein is included in the invention. This screening method comprises adding an agent to be tested to a system in which an index of the modification of G protein-coupled receptor protein in response to a physiological characteristic of the G protein-coupled receptor protein is measured making use of the thus constructed G protein-coupled receptor protein, and measuring the index. The following screening methods can be cited as illustrative examples of this measuring system. Also, examples of useful drugs to be tested include compounds or peptides which are conventionally known to have G protein-coupled receptor ligand activity but their ability to selectively modify activity of the novel G protein-coupled receptor protein is not clear, known compounds and peptides registered in chemical files but their various G protein-coupled receptor ligand activities are unknown, compounds obtained by the method such as combinatorial chemistry techniques (Terrett, N.K. *et al.* (1995), *Tetrahedron*, 51, 8135 - 8137) and random peptides prepared by employing a phage display (Felici, F. *et al.*

(1991), *J. Mol. Biol.*, 222, 301 - 310) or the like. In addition, culture supernatants of microorganisms, natural components originated from plants and marine organisms, animal tissue extracts and the like are also objects of the screening. Also useful are compounds or peptides obtained by chemically or biologically modifying a compound or peptide selected by the screening method of the invention.

3) Screening methods of ligands of the G protein-coupled receptor protein of the invention, namely compounds, peptides and antibodies which modify activity of the G protein-coupled receptor protein of the invention

a) A screening method which uses a ligand binding assay method

Compounds, peptides and antibodies which bind to the G protein-coupled receptor protein of the invention (generally referred to as ligand) can be screened by a ligand binding assay method. A cell membrane sample obtained after expression of the receptor protein or a purified sample of the receptor protein is prepared, and a ligand purified for use in the ligand binding assay is radiation-labeled (50 to 2,000 Ci/mmol). Buffer solution, ions, pH and the like assay conditions are optimized, and the receptor protein-expressed cell membrane sample or the purified receptor protein sample is incubated in the thus

optimized buffer for a predetermined period of time together with the radiation-labeled ligand. After the reaction, this is filtered through, e.g., a glass filter and washed with an appropriate amount of the buffer, and then the radioactivity remained on the filter (total binding amount) is measured using, e.g., a liquid scintillation counter. Nonspecific binding amount is measured by adding the unlabeled ligand in large excess in the reaction solution, and the specific binding amount is obtained by subtracting the nonspecific binding amount from the total binding amount. A ligand showing specific binding to the receptor protein-expressed cell membranes or the purified receptor protein can be selected as a ligand of the G protein-coupled receptor protein of the invention. In addition, a compound, peptide or antibody having agonist activity, or a compound, peptide or antibody having antagonist activity, of the G protein-coupled receptor protein can be screened making use of the binding inhibition of the thus obtained radioactive ligand as an index.

b) A screening method which uses a GTPγS binding method

Compounds, peptides and antibodies capable of modifying the activity of the G protein-coupled receptor protein of the invention can be screened by a GTPγS binding

method (Lazareno, S. and Birdsall, N.J.M. (1993), *Br. J. Pharmacol.*, 109, 1120 - 1127). Cell membranes obtained after expression of the receptor protein is mixed with 400 pM of GTPγS labeled with ^{35}S in a solution of 20 mM HEPES (pH 7.4), 100 mM NaCl, 10 mM $MgCl_2$ and 50 mM GDP. After incubation in the presence or absence of an agent to be tested, this is filtered through, e.g., a glass filter and then radioactivity of the bound GTPγS is measured using, e.g., a liquid scintillation counter. A compound, peptide or antibody having agonist activity of the G protein-coupled receptor protein can be screened making use, as an index, of the increased specific GTPγS binding in the presence of the drug to be tested. Also, a compound, peptide or antibody having antagonist activity of the G protein-coupled receptor protein can be screened making use, as an index, of the suppression of increase in the GTPγS binding by the thus obtained compound, peptide or antibody having agonist activity.

c) A screening method which uses changes in the intracellular Ca^{++} and cAMP concentrations

Many G protein-coupled receptor proteins induce increase in Ca^{++} and/or increase or decrease in cAMP concentration in the cells caused by an agonist stimulus. Accordingly, compounds, peptides and antibodies capable of

modifying the activity of the G protein-coupled receptor protein of the invention can be screened making use of the changes in the intracellular Ca^{++} or cAMP concentration. The Ca^{++} concentration is measured using fura2 and the like, and the cAMP concentration is measured using a commercially available cAMP assay kit (by Amersham, etc.).

Alternatively, it is possible to measure the Ca^{++} and cAMP concentrations indirectly, by detecting the transcription activity of a gene whose transcription amount is controlled depending on the Ca^{++} and cAMP concentrations. A sample such as a compound, a peptide, a tissue extract or the like is allowed to react for a predetermined period of time with cells in which the receptor protein is expressed or host cells in which the receptor protein is not expressed (control cells), and the Ca^{++} and cAMP concentrations are measured directly or indirectly. A compound, peptide or antibody having agonist activity can be screened making use, as an index, of the increase in Ca^{++} and/or increase or decrease in cAMP concentration in the receptor protein-expressed cells by comparing with the control cells. Also, a compound, peptide or antibody having antagonist activity of the G protein-coupled receptor protein can be screened making use, as an index, of the increase in Ca^{++} and/or increase or decrease in cAMP concentration caused by the thus

obtained compound, peptide or antibody having agonist activity.

d) A screening method which uses Microphysiometer

Upon various signal responses of cells, trace amount of hydrogen ions outflow into the extracellular moiety is detected. Most of this outflow of hydrogen ions occur when metabolites formed by the fuel consumption of cells to obtain energy for their responses are increased or when signals of the cells are transmitted directly to the hydrogen ion pump. Since the G protein-coupled receptor protein of the invention requires energy for its signal transmission, outflow of hydrogen ions occurs when the receptor is activated. Since changes in pH caused by such a trace outflow of hydrogen ions in a medium around cells can be detected by CYTOSENSOR Microphysiometer (Molecular Devices), it can be used for the detection of the activation energy consuming receptors.

A compound, a peptide, a tissue extract or the like is allowed to react for a predetermined period of time with cells in which the receptor protein is expressed or host cells in which the receptor protein is not expressed (control cells), and changes in the pH due to outflow of hydrogen ions are measured. A compound, peptide or antibody having agonist activity can be screened making

use, as an index, of the changes in pH caused by the outflow of hydrogen ions from the receptor protein-expressed cells by comparing with the control cells. Also, a compound, peptide or antibody having antagonist activity of the G protein-coupled receptor protein can be screened making use, as an index, of the changes in pH due to the outflow of hydrogen ions caused by the thus obtained compound, peptide or antibody having agonist activity.

A medicament which contains as the active ingredient a compound, peptide or antibody capable of significantly modifying the activity of the G protein-coupled receptor protein or a G protein-coupled receptor protein selected by the screening method is included in the invention.

The antibody, such as a polyclonal antibody or monoclonal antibody, which reacts with the G protein-coupled receptor protein of the invention can be obtained by directly administering the novel G protein-coupled receptor protein or a fragment of the G protein-coupled receptor protein to various animals. It can also be obtained by a DNA vaccine method (Raz, E. *et al.* (1994), *Proc. Natl. Acad. Sci. USA*, 91, 9519 - 9523; Donnelly, J.J. *et al.* (1996), *J. Infect. Dis.*, 173, 314 - 320) using a plasmid in which a gene which encodes the G protein-coupled receptor protein of the invention is introduced.

The polyclonal antibody is produced from sera or eggs of an animal (e.g., rabbit, rat, goat, fowl or the like) immunized and sensitized by emulsifying the G protein-coupled receptor protein or a fragment thereof in an appropriate adjuvant such as complete Freund's adjuvant and administering it by intraperitoneal, subcutaneous or intravenous injection. The polyclonal antibody thus produced from sera or eggs can be separated and purified by the usual protein isolation purification methods. Examples of such methods include centrifugation, dialysis, salting out with ammonium sulfate, and chromatographic techniques using carriers such as DEAE-cellulose, hydroxyapatite, protein A agarose and the like.

An active antibody fragment containing a part of the antibody, such as F(ab')2, Fab, Fab' or Fv, can be obtained by digesting the thus separated and purified antibody with a proteolytic enzyme such as pepsin, papain or the like in the usual way and subsequently separating and purifying it by the usual protein isolation purification methods.

It is possible for those skilled in the art to easily produce a monoclonal antibody by the cell fusion method of Kohler and Milstein (Kohler, G. and Milstein, C. (1975), *Nature*, 256, 495 - 497).

Mice are immunized by intraperitoneal, subcutaneous or intravenous inoculation of an emulsion prepared by

emulsifying the G protein-coupled receptor protein of the invention or a fragment thereof in an appropriate adjuvant such as complete Freund's adjuvant, several times repeatedly at intervals of several weeks. After final immunization, spleen cells are collected and fused with myeloma cells to prepare a hybridoma.

Myeloma cells having hypoxanthine-guanine phosphoribosyltransferase deficiency, thymidine kinase deficiency or the like marker, such as mouse myeloma cell strain P3X63Ag8.U1, are used as the myeloma cells for obtaining the hybridoma. Also, polyethylene glycol is used as the fusing agent. In addition, Eagle's minimum essential medium, Dulbecco's modified minimum essential medium, RPMI-1640 or the like generally used medium is optionally supplemented with 10 to 30% of fetal bovine serum and used as the medium for the preparation of the hybridoma. Fused strains are selected by the HAT selection method. Screening of hybridoma is carried out using a conventional method such as the culture supernatant by ELISA, immunohistological staining or the like or by the screening method described in the foregoing, and a hybridoma clone secreting the antibody of interest is selected. Also, monoclonal nature of the hybridoma is confirmed by repeating subcloning by means of limiting dilution analysis. When the thus obtained hybridoma is

cultured for 2 to 4 days in a medium or for 10 to 20 days in the abdominal cavity of a BALB/c mice pretreated with pristane, the antibody in an amount sufficient for purification is produced.

The thus produced monoclonal antibody can be separated and purified from the culture supernatant or ascites by the usual protein isolation purification methods. Examples of such methods include centrifugation, dialysis, salting out with ammonium sulfate, and chromatographic techniques using carriers such as DEAE-cellulose, hydroxyapatite, protein A agarose and the like. In addition, the monoclonal antibody or an antibody fragment containing a part thereof can also be produced by integrating entire portion or a part of a gene coding for the antibody into an expression vector and introducing into *Escherichia coli*, yeast or animal cells. An active antibody fragment containing a part of the antibody, such as F(ab')2, Fab, Fab' or Fv, can be obtained by digesting the thus separated and purified antibody with a proteolytic enzyme such as pepsin, papain or the like in the usual way and subsequently separating and purifying it by the usual protein isolation purification methods.

In addition, it is possible to obtain an antibody capable of reacting with the G protein-coupled receptor protein of the invention as single chain Fv or Fab by the

method of Clackson *et al.* or Zebedee *et al.* (Clackson, T. *et al.* (1991), *Nature*, 352, 624 - 628; Zebedee, S. *et al.* (1992), *Proc. Natl. Acad. Sci. USA*, 89, 3175 - 3179). It is also possible to obtain a human antibody by immunizing a transgenic mouse in which a mouse antibody gene is replaced by a human antibody gene (Lonberg, N. *et al.* (1994), *Nature*, 368, 856 - 859).

The medicament of the invention is characterized in that it has a novel pharmacological action to selectively control activity of the G protein-coupled receptor, and examples of the use of the medicament include central nervous system diseases which are induced by abnormalities of the G protein-coupled receptor activity (acceleration, reduction, denaturation and the like) or which express the abnormalities as complications.

The pharmaceutical preparation which contains a compound, peptide, antibody or antibody fragment capable of modifying activity of the G protein-coupled receptor protein of the invention, as the active ingredient, can be prepared using carriers, fillers and other additives generally used in the preparation of medicaments, in response to each type of the active ingredient.

Examples of its administration include oral administration in the form of tablets, pills, capsules, granules, fine granules, powders, oral solutions and the

like, and parenteral administration in the form of intravenous, intramuscular and the like injections, suppositories, percutaneous preparations, transmucosal absorption preparations and the like. Particularly, in the case of peptides which are digested in the stomach, intravenous injection or the like parenteral administration is desirable.

In the solid composition for use in the oral administration according to the present invention, one or more active substances are mixed with at least one inert diluent such as lactose, mannitol, glucose, microcrystalline cellulose, hydroxypropylcellulose, starch, polyvinyl pyrrolidone or aluminum magnesium metasilicate. In the usual way, the composition may contain additives other than the inert diluent, for example, a lubricant, a disintegrating agent, a stabilizing agent and a solubilizing or solubilization assisting agent. If necessary, tablets or pills may be coated with a sugar coating or a film of a gastric or enteric substance.

The liquid composition for oral administration includes emulsions, solutions, suspensions, syrups and elixirs and contains a generally used inert diluent such as purified water or ethanol. In addition to the inert diluent, this composition may also contain other additives

such as moistening agents, suspending agents, sweeteners, flavors and antiseptics.

The injections for parenteral administration includes aseptic aqueous or non-aqueous solutions, suspensions and emulsions. Examples of the diluent for use in the aqueous solutions and suspensions include distilled water for injection use and physiological saline. Examples of the diluent for use in the non-aqueous solutions and suspensions include propylene glycol, polyethylene glycol, plant oils (e.g., olive oil), alcohols (e.g., ethanol), polysorbate 80 and the like. Such a composition may further contain a moistening agent, an emulsifying agent, a dispersing agent, a stabilizing agent, a solubilizing or solubilization assisting agent, an antiseptic and the like. These compositions are sterilized for example by filtration through a bacteria retaining filter, blending of a germicide or irradiation. Alternatively, they may be used by firstly making into sterile solid compositions and dissolving them in sterile water or other sterile solvent for injection use prior to their use.

The dose is optionally decided by taking into consideration strength of each active ingredient selected by the screening method described in the foregoing and symptoms, age, sex and the like of each patient to be administered.

Brief Description of the Drawings

Fig. 1 shows alignment of amino acid sequences of SREB1, SREB2 and SREB3.

Fig. 2 shows a result of Northern analysis of SREB1 in human organs.

Fig. 3 shows a result of Northern analysis of SREB1 in each region of human brain.

Fig. 4 shows a result of Northern analysis of SREB2 in human organs.

Fig. 5 shows a result of Northern analysis of SREB2 in each region of human brain.

Fig. 6 shows a result of Northern analysis of SREB3 in human organs.

Fig. 7 shows a result of Northern analysis of SREB3 in each region of human brain.

Fig. 8 shows a result confirming expression of SREB1, SREB2 or SREB3 protein.

Fig. 9 shows binding activity of anti-3LO antibody for SREB1, SREB2 or SREB3.

Fig. 10 shows binding activity of anti-C24 antibody for SREB1.

Fig. 11 shows pCRE-Luc derived luciferase activity in cells in which SREB1, SREB2 or SREB3 was introduced.

Fig. 12 shows pSRE-Luc derived luciferase activity in cells in which SREB1, SREB2 or SREB3 was introduced.

Best Mode for Carrying Out the Invention

In order to disclose the invention further illustratively, Examples are described in the following, but the invention is not limited to these Examples. In this connection, unless otherwise noted, they can be carried out in accordance with known methods (Maniatis, T. *et al.* (1982): "Molecular Cloning - A Laboratory Manual", Cold Spring Harbor Laboratory, NY).

(Example 1) Isolation of genes coding for the novel G protein-coupled receptor family proteins

Full length cDNA coding for the G protein-coupled receptor family protein (SREB1, SREB2 or SREB3) of the invention was obtained by RT-PCR using human brain origin poly A^+ RNA (Clontech) as the template.

In the amplification of the novel G protein-coupled receptor human SREB1, 5′-AAAATCTAGA CGCGATGGCGAACGCGAGCGA-3′ (Sequence No. 7) was used as the forward primer, and 5′-AAAATCTAGA GTCTATGTGGCGGGGCCTCCC-3′ (Sequence No. 8) as the reverse primer (*Xba*I site is added to each 5′ terminus). RT-PCR was carried out using Pfu DNA polymerase (Stratagene) and by repeating a cycle of 98°C (20 seconds)/64°C (30 seconds)/74°C (3 minutes) 34 times in the

presence of 5% formamide. As the result, a DNA fragment of about 1.2 kbp was amplified. This fragment was digested with *Xba*I and then cloned using pCEP4 plasmid (Invitrogen). Since the pCEP4 plasmid contains CMV promoter which shows strong promoter activity in animal cells, it can be used in expressing recombinant proteins in animal cells.

Nucleotide sequence of the thus obtained clone was analyzed by the dideoxy terminator method using ABI377 DNA Sequencer (Applied Biosystems). The thus revealed sequence is shown in Sequence No. 1 of the Sequence Listing.

This sequence contains an open reading frame of 1,125 bases (from the 1st position to the 1125th position of Sequence No. 1). An amino acid sequence (375 amino acids) deduced from the open reading frame is shown in Sequence No. 2 of the Sequence Listing. Since the deduced amino acid sequence contains seven hydrophobic regions considered to be the transmembrane domains which is a characteristic of the G protein-coupled receptor, it was found that this gene encodes the G protein-coupled receptor.

In the amplification of the novel G protein-coupled receptor human SREB2, 5′-AAAATCTAGA TCTATGGCGAACTATAGCCATGCA-3′ (Sequence No. 9) was used as the forward primer, and 5′-AAAATCTAGA AAGGCTAAAGATTTACAGATGCTCC-3′ (Sequence No. 10) as the reverse primer (*Xba*I site is added to each 5′ terminus).

RT-PCR was carried out using Pfu DNA polymerase (Stratagene) and by repeating a cycle of 96°C (20 seconds)/54°C (30 seconds)/74°C (3 minutes) 34 times. As the result, a DNA fragment of about 1.2 kbp was amplified. This fragment was digested with *Xba*I and then cloned using pCEP4 plasmid (Invitrogen). Nucleotide sequence of the thus obtained clone was analyzed by the dideoxy terminator method using ABI377 DNA Sequencer (Applied Biosystems). The thus revealed sequence is shown in Sequence No. 3 of the Sequence Listing.

This sequence contains an open reading frame of 1,110 bases (from the 1st position to the 1110th position of Sequence No. 3). An amino acid sequence (370 amino acids) deduced from the open reading frame is shown in Sequence No. 4 of the Sequence Listing. Since the deduced amino acid sequence contains seven hydrophobic regions considered to be the transmembrane domains which is a characteristic of the G protein-coupled receptor, it was found that this gene encodes the G protein-coupled receptor.

In the amplification of the novel G protein-coupled receptor human SREB3, 5′-AAAATCTAGA GTATGGCCAACACTACCGGAGAG-3′ (Sequence No. 11) was used as the forward primer, and 5′-AAAATCTAGA CCTGTCTGCCTACCAGCCTGC-3′ (Sequence No. 12) as the reverse primer (*Xba*I site is added to each 5′ terminus). RT-PCR

was carried out using Pfu DNA polymerase (Stratagene) and by repeating a cycle of 98°C (20 seconds)/62°C (30 seconds)/74°C (3 minutes) 34 times in the presence of 5% formamide. As the result, a DNA fragment of about 1.2 kbp was amplified. This fragment was digested with *Xba*I and then cloned using pCEP4 plasmid (Invitrogen). Nucleotide sequence of the thus obtained clone was analyzed by the dideoxy terminator method using ABI377 DNA Sequencer (Applied Biosystems). The thus revealed sequence is shown in Sequence No. 5 of the Sequence Listing.

This sequence contains an open reading frame of 1,119 bases (from the 1st position to the 1119th position of Sequence No. 5). An amino acid sequence (373 amino acids) deduced from the open reading frame is shown in Sequence No. 6 of the Sequence Listing. Since the deduced amino acid sequence contains seven hydrophobic regions considered to be the transmembrane domains which is a characteristic of the G protein-coupled receptor, it was found that this gene encodes the G protein-coupled receptor.

Homology of the novel G protein-coupled receptor SREB family (SREB1, SREB2 or SREB3) with a known G protein-coupled receptor family is 25% or less, respectively.

On the other hand, homology of SREB1 with SREB2 is 52%, homology of SREB1 with SREB3 is 52% and homology of SREB2 with SREB3 is 63%, which are significantly higher

than the homology with known G protein-coupled receptors (Fig. 1). This fact shows that the G protein-coupled receptors SREB1, SREB2 and SREB3 of the invention form a novel G protein-coupled receptor family independent of the known G protein-coupled receptors.

(Example 2) Expression distribution of human novel G protein-coupled receptor family genes in tissues

Expression distribution of the G protein-coupled receptor genes of the invention was analyzed by the northern blot hybridization method. A cDNA fragment (from the 722nd position to the 1054th position in Sequence No. 1) was used as the probe of human SREB1. Poly A^+ RNA (2 μg) originated from each of human organs was blotted on a membrane (Clontech), and its hybridization with the probe was carried out at 42°C (18 hours) in a solution containing 50% formamide, 5 x SSPE, 10 x Denhardt's solution, 2% SDS and 100 μg/ml denatured salmon sperm DNA. The membrane was finally washed twice (65°C for 30 minutes) with a solution containing 0.2 x SSC and 0.1% SDS. As shown in Fig. 2, when the northern analysis was carried out on each of human organs (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, large intestine and peripheral leukocyte), 3 kb of mRNA was detected in the brain, ovary, testis, heart and prostate, and 3 kb and 2.3 kb mRNA in the

peripheral leukocyte. Also, a signal of 3 kb was slightly detected in the pancreas, too. In addition, the northern analysis was also carried out on each of the regions of the human brain (amygdala, caudate nucleus, corpus callosum, hippocampus, substania nigra, subthalamic nucleus, thalamus, cerebellum, cerebral cortex, medulla, spinal cord, occipital lobe, frontal lobe, temporal lobe and putamen). Since the 3 kb mRNA of the G protein-coupled receptor human SREB1 gene of the invention was detected in all of the examined human brain regions, it was found that it is expressed broadly in the human brain (Fig. 3).

A cDNA fragment (from the 558th position to the 888th position in Sequence No. 3) was used as the probe of human SREB2. When the northern analysis was carried out under the same conditions, 3.2 kb mRNA was detected in the brain, and 2.4 kb, 3.5 kb and 6.3 kb mRNA in the testis, as shown in Fig. 4. Also, the signal of 3.5 kb was detected in the placenta and spleen, and the signal of 3.2 kb in small intestine, all slightly. Among regions in the brain, the 3.2 kb mRNA of the G protein-coupled receptor human SREB2 gene of the invention was abundantly detected in the amygdala, caudate nucleus, hippocampus, substania nigra, subthalamic nucleus, thalamus, cerebellum, cerebral cortexes and putamen, but not so much in the corpus callosum, medulla and spinal cord. In addition, a signal

of 7.8 kb was slightly detected in each of the grain regions (Fig. 5)

A cDNA fragment (from the 1st position to the 652nd position in Sequence No. 5) was used as the probe of human SREB3. When the northern analysis was carried out under the same conditions, 4 kb and 5.1 kb mRNA was detected in the brain, and 4 kb, 5.1 kb and 9.7 kb mRNA in the ovary, as shown in Fig. 6. The G protein-coupled receptor human SREB3 gene of the invention was detected in each region of the brain as signals of mainly 4 kb, 5.1 kb and slightly 9.7 kb, and the 4 kb mRNA was detected in the amygdala, hippocampus, subthalamic nucleus, cerebellum and cerebral cortex, and the 5.1 kb mRNA in the substania nigra, subthalamic nucleus and spinal cord, relatively abundantly (Fig. 7).

The above results showed that the G protein-coupled receptor family genes SREB1, SREB2 and SREB3 of the invention are expressed mainly in the central nervous system and urinary organ/reproductive organ system.

(Example 3) Confirmation of the expression of the novel human G protein-coupled receptor family proteins

pCEP4 (Invitrogen) was used as the expression vector for expressing human SREB1, SREB2 or SREB3. In this case, in order to fuse a FLAG epitope as a marker sequence with the N terminus of human SREB1, SREB2 or SREB3,

ATGGACTACAAGGACGACGATGACAAGGGGATCCTG (Sequence No. 13) was inserted into the 5′ terminus of the protein coding sequence of SREB1, SREB2 or SREB3. The thus constructed plasmids were named pCEP4-FL-SREB1, pCEP4-FL-SREB2 and pCEP4-FL-SREB3, respectively. By the use of these plasmids, a polypeptide in which a sequence Met Asp Tyr Lys Asp Asp Asp Asp Lys Gly Ile Leu (Sequence No. 14) is fused with the N terminus of the polypeptide of SREB1, SREB2 or SREB3 is expressed.

A 1 x 10^6 cells portion of 293-EBNA (Invitrogen) was inoculated into a 10 cm Petri dish and cultured for 1 day, and then gene transfer of 8 μg of pCEP4-FL-SREB1, pCEP4-FL-SREB2, pCEP4-FL-SREB3 or pCEP4-FL (vector alone) was carried out using FuGENE6 (Boeringer Mannheim). After the gene transfer, the cells were cultured for 1 day, harvested, washed, suspended in 20 mM of Tris-HCl (pH 7.4)/150 mM NaCl/Complete™ (Boeringer Mannheim) and then homogenized using Polytron. The homogenate was mixed with Triton X-100, Digitonin and sodium cholate to final concentrations of 0.2%, 0.1% and 0.2% and then solubilized by incubating at 4°C for 2 hours. Immunoprecipitation of the FLAG epitope fusion protein from the thus solubilized sample was effected using M2-agarose (Sigma). The immune precipitate was eluted with 200 μM FLAG peptide/20 mM Tris-HCl (pH 7.4)/150 mM NaCl. The eluted sample was

concentrated, subjected to electrophoresis using SDS/10%-20% acrylamide gel (Daiichi Pure Chemicals) and then transferred on a PVDF membrane using a blotting apparatus. The PVDF membrane after the transfer was subjected to blocking and then allowed to react with a mouse anti-FLAG monoclonal antibody (M2; Sigma) and a horseradish peroxidase-labeled rabbit anti-mouse IgG polyclonal antibody (Zymed) in that order. After the reaction, expression of SREB1, SREB2 or SREB3 protein was confirmed using ECL Western Blotting Detection System (Amersham-Pharmacia) (Fig. 8).

The protein capable of reacting with the anti-FLAG antibody was not present in the cells in which pCEP4-FL was introduced but detected in the cells in which pCEP4-FL-SREB1, pCEP4-FL-SREB2 or pCEP4-FL-SREB3 was introduced as a band of 35 to 45 kDa. Estimated molecular weights of human SREB1, human SREB2 and human SREB3 were 39.8 kDa, 42.0 kDa and 41.5 kDa, respectively, and their bands were found at positions of almost expected molecular weights. In addition, a band of 65 to 75 kDa considered to be a dimer was detected in the case of human SREB1.

(Example 4) Isolation of gene coding for rat SREB1 (rSREB1), rat SREB2 (rSREB2) or rat SREB3 (rSREB3) protein

Complete length cDNA coding for rSREB2, rSREB2 or rSREB3 was obtained by RT-PCR using rat brain origin poly A^+ RNA (Clontech) as the template.

In the amplification of rSREB1, 5′-AAAATCTAGACGGCGATGGCGAACGCTAGTGA-3′ (Sequence No. 15) was used as the forward primer, and 5′-AAAATCTAGA CACTTTGAGAGTCTTGTGAAGGC-3′ (Sequence No. 16) as the reverse primer (*Xba*I site is added to each 5′ terminus). Amplification, cloning and nucleotide sequence determination of cDNA were carried out by the same methods of Example 1. The thus revealed sequence is shown in Sequence No. 21 of the Sequence Listing.

This sequence contains an open reading frame of 1,131 bases (from the 1st position to the 1131st position of Sequence No. 21). An amino acid sequence (377 amino acids) deduced from the open reading frame is shown in Sequence No. 22 of the Sequence Listing. Since the deduced amino acid sequence coincided in 97% frequency with the human SREB1, it was found that this gene encodes rSREB1.

In the amplification of rSREB2, 5′-AAAATCTAGATCTATGGCGAACTATAGCCATGC-3′ (Sequence No. 17) was used as the forward primer, and 5′-AAAATCTAGA AAGGCTAAAGATTTACAGATGCTCC-3′ (Sequence No. 18) as the

reverse primer (*Xba*I site is added to each 5′ terminus). Amplification, cloning and nucleotide sequence determination of cDNA were carried out by the same methods of Example 1. The thus revealed sequence is shown in Sequence No. 23 of the Sequence Listing.

This sequence contains an open reading frame of 1,110 bases (from the 1st position to the 1110th position of Sequence No. 23). An amino acid sequence (370 amino acids) deduced from the open reading frame is shown in Sequence No. 24 of the Sequence Listing. Since the deduced amino acid sequence coincided in 100% frequency with the human SREB2, it was found that this gene encodes rSREB2.

In the amplification of rSREB3, 5′-AAAATCTAGACAAATACTGAACTGGCCGATCCCC-3′ (Sequence No. 19) was used as the forward primer, and 5′-AAAATCTAGA TGTTGGCCCCAGTATGGTGATCAT-3′ (Sequence No. 20) as the reverse primer (*Xba*I site is added to each 5′ terminus). Amplification, cloning and nucleotide sequence determination of cDNA were carried out by the same methods of Example 1. The thus revealed sequence is shown in Sequence No. 25 of the Sequence Listing.

This sequence contains an open reading frame of 1,119 bases (from the 1st position to the 1119th position of Sequence No. 25). An amino acid sequence (373 amino acids) deduced from the open reading frame is shown in Sequence

No. 26 of the Sequence Listing. Since the deduced amino acid sequence coincided in 99% frequency with the human SREB3, it was found that this gene encodes rSREB3.

(Example 5) Preparation of antibody for human SREB1

A partial amino acid sequence of human SREB1 was fused with glutathione-S-transferase (GST) and used as the immunization antigen for the preparation of antibody for human SREB1. Illustratively, a cDNA fragment corresponding to a region of from the 208th position to the 282nd position (3LO) and a region of from the 351st position to the 375th position (C24) of the human SREB1 amino acid sequence (Sequence No. 2) was amplified by PCR in an way to bind cleavage sites of restriction enzymes *Bam*HI and *Xho*I, and inserted between *Bam*HI and *Xho*I sites of GST Gene Fusion Vector (pGEX-5X-1: Amersham-Pharmacia). Competent cells of an *Escherichia coli* strain BL21(DE3)pLysS (Novagen) were transformed with the thus constructed plasmid. By culturing the transformant and inducing expression of the gene with 1 mM IPTG, a GST-3LO fusion protein and a GST-C24 fusion protein were expressed in the *E. coli* cells. The GST-3LO and GST-C24 were purified from disrupted *E. coli* cells using Glutathione Sepharose 4B (Amersham-Pharmacia) in accordance with the instruction attached thereto.

The thus purified GST-3LO fusion protein was mixed with the same amount of Freund's complete adjuvant (CalBioChem) and emulsified, and the emulsion was administered to a female white Leghorn (140 days of age) at around the bursa of Fabricius. The initial dose was 1 mg, and it was administered thereafter in 0.5 mg portions 4 times at intervals of 2 weeks. After the final immunization, eggs were collected, the yolk of eggs was diluted with physiological saline and defatted using dextran sulfate, and then IgY was purified using DEAE Sepharose (Amersham-Pharmacia) to obtain anti-3LO antibody. Also, the purified GST-C24 fusion protein was mixed with the same amount of TiterMax Gold (CytRX) and emulsified, and the emulsion was administered under the dorsal skin of a Japanese white rabbit (6 weeks of age). Its initial dose was 1 mg, and it was administered thereafter in 0.5 mg portions 2 times at intervals of 2 weeks. After the final immunization, blood was collected, and IgG was purified from the serum using Protein G Sepharose (Amersham-Pharmacia) in accordance with the instruction attached thereto, thereby obtaining anti-C24 antibody.

Since the anti-3LO antibody uses a region of from the 208th position to the 282nd position of the human SREB1 amino acid sequence (Sequence No. 2) as the antigen and this partial amino acid sequence contains a large number of

sequences common to SREB1, SREB2 and SREB3 (cf. Fig. 1), there is a possibility that the anti-3LO antibody commonly recognizes SREB1, SREB2 and SREB3. Also, since the anti-C24 antibody uses a region of from the 351st position to the 375th position of the human SREB1 amino acid sequence (Sequence No. 2) as the antigen and this partial amino acid sequence is a sequence in which SREB2 and 3 are not present but SREB1 alone is present (cf. Fig. 1), there is a possibility that the anti-C24 antibody recognizes only SREB1. In consequence, in order to confirm the specificity of anti-3LO antibody and anti-C24 antibody, Western blotting was carried out using the immune precipitate of anti-FLAG antibody of 293-EBNA in which SREB1, SREB2 or SREB3 was expressed, prepared in Example 3, and the anti-3LO antibody and anti-C24 antibody.

Illustratively, each sample was subjected to electrophoresis using SDS/10%-20% acrylamide gel (Daiichi Pure Chemicals) and then transferred on a PVDF membrane using a blotting apparatus. The PVDF membrane after the transfer was subjected to blocking and then allowed to react with 10 μg/ml of the anti-3LO antibody and a horseradish peroxidase-labeled rabbit anti-chicken IgG polyclonal antibody (Zymed) in that order or with 10 μg/ml of the anti-C24 antibody and a horseradish peroxidase-labeled goat anti-rabbit IgG polyclonal antibody (MBL) in

that order. After the reaction, color development was carried out using ECL Western Blotting Detection System (Amersham-Pharmacia). A band reacting with the anti-3LO antibody was detected at the same position of the anti-FLAG antibody of Example 3 in cells in which pCEP4-FL-SREB1, pCEP4-FL-SREB2 or pCEP4-FL-SREB3 (Fig. 9) was introduced. Also, a band reacting with the anti-C24 antibody was detected at the same position of the anti-FLAG antibody of Example 3 only in the cells in which pCEP4-FL-SREB1 was introduced (Fig. 10).

Based on the above results, it was confirmed that the anti-3LO antibody is an antibody which recognizes SREB1, SREB2 or SREB3, and the anti-C24 antibody is an antibody which recognizes only SREB1. The use of these antibodies has rendered possible the detection of natural SREB1, SREB2 or SREB3 by the method such as the Western blotting, immunohistological staining or the like.

(Example 6) Evaluation of transcription activity via cAMP-response element (CRE) or serum response element (SRE) in human SREB1-, SREB2- or SREB3-introduced cells

Increase in the transcription activity mediated by CRE or SRE is induced by the activation of the intracellular information transmission system of various G protein-coupled receptors (Lolait, S.J. *et al.* (1992),

Nature, 357, 336 - 339; Hoeltzel, W.L. *et al.* (1997), *Am. J. Physiol.*, 273, C2037 - C2045; An, S. *et al.* (1998), *J. Biol. Chem.*, 273, 7906 - 7910). Also, it is known that the intracellular information transmission system of G protein-coupled receptors is partially activated via a certain transitional active conformation even in the absence of agonist (Kenakin, T. (1995), *Trens. Pharmacol. Sci.*, 16, 188 - 192). Accordingly, if changes in the CRE- or SRE-mediated transcription activity in SREB1-, SREB2- or SREB3-introduced cells are found even in the absence of agonist, it can be confirmed that the G protein-coupled receptor is functional and that activation of the G protein-coupled receptor intracellular information transmission system leads to the CRE- and SRE-mediated transcription activity.

Using pEF-BOS (Mizushima, S. and Nagata, S. (1990), *Nucleic Acids Res.*, 18, 5322) as the expression vector for expressing human SREB1, SREB2 or SREB3, pEF-BOS-SREB1, pEF-BOS-SREB2 and pEF-BOS-SREB3 were prepared. A 8 x 10^4 cells portion of 293-EBNA (Invitrogen) was inoculated into a 24-well plate and cultured for 1 day, and then gene transfer of 250 ng of pEF-BOS-SREB1, pEF-BOS-SREB2, pEF-BOS-SREB3 or pEF-BOS (vector alone) was carried out together with 25 ng of a CRE-reporter plasmid pCRE-Luc (Stratagene) or an SRE-reporter plasmid pSRE-Luc (Stratagene), using FuGENE6 (Boeringer Mannheim) (3 wells for each). After the gene

transfer, the cells were lysed at every 12 hours using PicaGene Cell Culture Lysis Reagent Luc (Nippon Gene), and the activity of luciferase produced from each reporter plasmid was measured using PicaGene Luminescence Kit (Nippon Gene).

The luciferase activity in the SREB1-, SREB2- or SREB3-introduced cells after 24 hours of the gene transfer was treated as a relative activity to the luciferase activity of the vector alone introduced cells (control) (the control was defined as 1), with the results shown in Fig. 11 (pCRE-Luc derived luciferase activity) and Fig. 12 (pSRE-Luc derived luciferase activity). The CRE-mediated transcription activity increased most sharply in the SREB1-introduced cells and also increased significantly in the SREB2- and SREB3-introduced cells in comparison with the control. On the other hand, the SRE-mediated transcription activity increased most sharply in the SREB2-introduced cells and also increased significantly in the SREB1- and SREB3-introduced cells in comparison with the control.

It was revealed by these results that the SREB1, SREB2 and SREB3 are functional receptors, and activation of the intracellular information transmission system of these G protein-coupled receptors leads to the increase in the CRE- or SRE-mediated transcription activity.

Industrial Applicability

Novel G protein-coupled receptor family proteins SREB1, SREB2 and SREB3 expressing in the central nervous system, genes coding for these proteins, vectors containing these genes, host cells containing these vectors and methods for producing these G protein-coupled receptor proteins were provided by the present invention.

Also, it rendered possible to screen new medicaments, particularly new therapeutic agents for central nervous system diseases, through the screening of compounds, peptides and antibodies capable of modifying activities of the G protein-coupled receptor proteins of the invention by allowing the G protein-coupled receptors to contact with drugs to be tested.

Regarding the medicament of the invention which contains, as the active ingredient, a compound, peptide or antibody capable of specifically modifying activity of the G protein-coupled receptor proteins expressing in the central nervous system, its usefulness as therapeutic agents and the like for functional/organic diseases of the central nervous system. Also, since the G protein-coupled receptor family proteins of the invention are expressed not only in the central nervous system but also in the urinary organ/reproductive organ system, usefulness as therapeutic drugs and the like for diseases related to the urinary

organ/reproductive organ system can be expected from the medicament which contains, as the active ingredient, a compound, peptide or antibody capable of specifically modifying their activities. In addition, since a member of the G protein-coupled receptors of the invention, such as SREB1 protein, is expressed not only in the central nervous system and urinary organ/reproductive organ system but also in the heart and peripheral leukocytes, a medicament which contains, as the active ingredient, a compound, peptide or antibody capable of specifically modifying the activity of SREB1 protein can be expected for its usefulness as therapeutic drugs and the like for circulatory system diseases and immune inflammation system diseases, in addition to central diseases and diseases related to the urinary organ/reproductive organ system.

The novel G protein-coupled receptor family SREB1, SREB2 or SREB3 of the invention has markedly high conservation ratio of amino acids in human and rat. This conservation ratio is most highest among the existing G protein-coupled receptor families, which seems to show that the novel G protein-coupled receptor family of SREB1, SREB2 and SREB3 is taking important roles in the living body, particularly a physiological role in the central nervous system. Also, since their amino acid sequences have a conversation ratio of 97% or more in human and rat, it is

considered that almost no interspecies differences are present regarding activities of drugs which act upon the novel G protein-coupled receptor family SREB1, SREB2 or SREB3. In consequence, when the G protein-coupled receptor protein of the invention itself or a compound or protein obtained by a screening using the receptor is developed as a medicament, the receptor has an advantage in that animal experiments using rats, for example, can be carried out in advance, prior to testing pharmacological effects on human, and is useful in terms that clinical data on human can be easily predicted from the animal experiment data.

Since expression of the G protein-coupled receptor proteins of the invention in organs and changes thereof can be detected by the method such as ELISA, radioimmunoassay, the Western blotting and the like using the antibodies, these antibodies for the novel G-protein coupled receptor proteins are useful as diagnostic agents. In addition, the antibodies capable of modifying activities of the novel G protein-coupled receptor proteins are useful as therapeutic drugs for diseases in which the novel G protein-coupled receptor proteins are involved and also as tools for the separation and purification of the receptor proteins.

Claims

1. A G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26, or a G protein-coupled receptor protein as an equivalent to said protein.

2. A G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26.

3. A gene which has a nucleotide sequence coding for the G protein-coupled receptor protein described in claim 1.

4. A vector which contains the gene described in claim 3.

5. A host cell which contains the vector described in claim 4.

6. A method for producing the G protein-coupled receptor protein described in claim 1 or 2, or a G protein-coupled receptor protein as an equivalent to said protein, which comprises using the host cell described in claim 5.

7. A method for screening a medicament acting on the G protein-coupled receptor protein described in claim 1 or 2, which comprises allowing said G protein-coupled receptor protein to contact with a compound to be tested.

8. An antibody or a fragment thereof for the G protein-coupled receptor protein described in claim 1 or 2 or a partial peptide thereof.

Abstract

This invention belongs to the genetic engineering field, and provides novel G protein-coupled receptor family proteins SREB1, SREB2 and SREB3 expressed in the central nervous system, genes coding for said proteins, vectors containing said genes, host cells containing said vectors, processes for producing said G protein-coupled receptor proteins, screening methods using said G protein-coupled receptor proteins, antibodies for said G protein-coupled receptor proteins, and screening methods using said antibodies.

Representative method for obtaining the G protein-coupled receptor proteins of the present invention:

The reverse transcriptase-polymerase chain reaction (to be referred to as RT-PCR hereinafter) is used for obtaining the G protein-coupled receptor proteins of the present invention. mRNA is extracted from human or rat brain tissue or brain-derived cells. Then, using the mRNA as the template and using two primers interposing the entire portion or a part of the G protein-coupled receptor protein translation region, RT-PCR is carried out to obtain cDNA corresponding to the G protein-coupled receptor protein or a part thereof. Then, the resulting cDNA of the novel G protein-coupled receptor protein or a part thereof

is ligated into an appropriate expression vector and expressed in a host cell to produce said G protein-coupled receptor protein.

Abstract

This invention belongs to the genetic engineering field, and provides novel G protein-coupled receptor family proteins SREB1, SREB2 and SREB3 expressed in the central nervous system, genes coding for these proteins, screening methods using these proteins and so on.

As one of the methods for obtaining the G protein-coupled receptor proteins of the present invention, RT-PCR is carried out using mRNA extracted from human or rat brain tissue or brain-derived cells as the template and using two primers interposing the entire portion or a part of the G protein-coupled receptor protein translation region, thereby obtaining cDNA corresponding to the G protein-coupled receptor protein or a part thereof, and the cDNA is integrated into an appropriate expression vector and expressed in a host cell.

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180 185 190

Leu Ile Leu Leu Ala Thr Gln Leu Val Tyr Leu Lys Leu Ile Phe Phe
195 200 205

Val His Asp Arg Arg Lys Met Lys Pro Val Gln Phe Val Ala Ala Val
210 215 220

Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Ser Gly Gln Ala Ala
225 230 235 240

Ala Asn Trp Leu Ala Gly Phe Gly Arg Gly Pro Thr Pro Pro Thr Leu
245 250 255

Leu Gly Ile Arg Gln Asn Ala Asn Thr Thr Gly Arg Arg Arg Leu Leu
260 265 270

Val Leu Asp Glu Phe Lys Met Glu Lys Arg Ile Ser Arg Met Phe Tyr
275 280 285

Ile Met Thr Phe Leu Phe Leu Thr Leu Trp Gly Pro Tyr Leu Val Ala
290 295 300

Cys Tyr Trp Arg Val Phe Ala Arg Gly Pro Val Val Pro Gly Gly Phe
305 310 315 320

Leu Thr Ala Ala Val Trp Met Ser Phe Ala Gln Ala Gly Ile Asn Pro
325 330 335

Phe Val Cys Ile Phe Ser Asn Arg Glu Leu Arg Arg Cys Phe Ser Thr
340 345 350

Thr Leu Leu Tyr Cys Arg Lys Ser Arg Leu Pro Arg Glu Pro Tyr Cys
355 360 365

Val Ile
370

<210> 5
<211> 1122
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1119)
<223> SREB3

<400> 5
atg gcc aac act acc gga gag cct gag gag gtg agc ggc gct ctg tcc 48
Met Ala Asn Thr Thr Gly Glu Pro Glu Glu Val Ser Gly Ala Leu Ser
1 5 10 15

cca ccg tcc gca tca gct tat gtg aag ctg gta ctg ctg gga ctg att 96
Pro Pro Ser Ala Ser Ala Tyr Val Lys Leu Val Leu Leu Gly Leu Ile
20 25 30

atg tgc gtg agc ctg gcg ggt aac gcc atc ttg tcc ctg ctg gtg ctc 144
Met Cys Val Ser Leu Ala Gly Asn Ala Ile Leu Ser Leu Leu Val Leu
35 40 45

aag gag cgt gcc ctg cac aag gct cct tac tac ttc ctg ctg gac ctg 192
Lys Glu Arg Ala Leu His Lys Ala Pro Tyr Tyr Phe Leu Leu Asp Leu
50 55 60

tgc ctg gcc gat ggc ata cgc tct gcc gtc tgc ttc ccc ttt gtg ctg 240
Cys Leu Ala Asp Gly Ile Arg Ser Ala Val Cys Phe Pro Phe Val Leu
65 70 75 80

gct tct gtg cgc cac ggc tct tca tgg acc ttc agt gca ctc agc tgc 288
Ala Ser Val Arg His Gly Ser Ser Trp Thr Phe Ser Ala Leu Ser Cys
85 90 95

aag att gtg gcc ttt atg gcc gtg ctc ttt tgc ttc cat gcg gcc ttc 336
Lys Ile Val Ala Phe Met Ala Val Leu Phe Cys Phe His Ala Ala Phe
100 105 110

atg ctg ttc tgc atc agc gtc acc cgc tac atg gcc atc gcc cac cac 384
Met Leu Phe Cys Ile Ser Val Thr Arg Tyr Met Ala Ile Ala His His
115 120 125

cgc ttc tac gcc aag cgc atg aca ctc tgg aca tgc gcg gct gtc atc 432
Arg Phe Tyr Ala Lys Arg Met Thr Leu Trp Thr Cys Ala Ala Val Ile
130 135 140

tgc atg gcc tgg acc ctg tct gtg gcc atg gcc ttc cca cct gtc ttt 480
Cys Met Ala Trp Thr Leu Ser Val Ala Met Ala Phe Pro Pro Val Phe
145 150 155 160

gac gtg ggc acc tac aag ttt att cgg gag gag gac cag tgc atc ttt 528
Asp Val Gly Thr Tyr Lys Phe Ile Arg Glu Glu Asp Gln Cys Ile Phe
165 170 175

gag cat cgc tac ttc aag gcc aat gac acg ctg ggc ttc atg ctt atg 576

```
Glu His Arg Tyr Phe Lys Ala Asn Asp Thr Leu Gly Phe Met Leu Met
                180                 185                 190

ttg gct gtg ctc atg gca gct acc cat gct gtc tac ggc aag ctg ctc   624
Leu Ala Val Leu Met Ala Ala Thr His Ala Val Tyr Gly Lys Leu Leu
        195                 200                 205

ctc ttc gag tat cgt cac cgc aag atg aag cca gtg cag atg gtg cca   672
Leu Phe Glu Tyr Arg His Arg Lys Met Lys Pro Val Gln Met Val Pro
    210                 215                 220

gcc atc agc cag aac tgg aca ttc cat ggt ccc ggg gcc acc ggc cag   720
Ala Ile Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Thr Gly Gln
225                 230                 235                 240

gct gct gcc aac tgg atc gcc ggc ttt ggc cgt ggg ccc atg cca cca   768
Ala Ala Ala Asn Trp Ile Ala Gly Phe Gly Arg Gly Pro Met Pro Pro
                245                 250                 255

acc ctg ctg ggt atc cgg cag aat ggg cat gca gcc agc cgg cgg cta   816
Thr Leu Leu Gly Ile Arg Gln Asn Gly His Ala Ala Ser Arg Arg Leu
            260                 265                 270

ctg ggc atg gac gag gtc aag ggt gaa aag cag ctg ggc cgc atg ttc   864
Leu Gly Met Asp Glu Val Lys Gly Glu Lys Gln Leu Gly Arg Met Phe
        275                 280                 285

tac gcg atc aca ctg ctc ttt ctg ctc ctc tgg tca ccc tac atc gtg   912
Tyr Ala Ile Thr Leu Leu Phe Leu Leu Leu Trp Ser Pro Tyr Ile Val
    290                 295                 300

gcc tgc tac tgg cga gtg ttt gtg aaa gcc tgt gct gtg ccc cac cgc   960
Ala Cys Tyr Trp Arg Val Phe Val Lys Ala Cys Ala Val Pro His Arg
305                 310                 315                 320

tac ctg gcc act gct gtt tgg atg agc ttc gcc cag gct gcc gtc aac  1008
Tyr Leu Ala Thr Ala Val Trp Met Ser Phe Ala Gln Ala Ala Val Asn
                325                 330                 335

cca att gtc tgc ttc ctg ctc aac aag gac ctc aag aag tgc ctg agg  1056
Pro Ile Val Cys Phe Leu Leu Asn Lys Asp Leu Lys Lys Cys Leu Arg
            340                 345                 350

act cac gcc ccc tgc tgg ggc aca gga ggt gcc ccg gct ccc aga gaa  1104
Thr His Ala Pro Cys Trp Gly Thr Gly Gly Ala Pro Ala Pro Arg Glu
        355                 360                 365

ccc tac tgt gtc atg tga                                         1122
Pro Tyr Cys Val Met
    370
```

<210> 6
<211> 373
<212> PRT
<213> Homo sapiens

<400> 6

Met Ala Asn Thr Thr Gly Glu Pro Glu Glu Val Ser Gly Ala Leu Ser
1 5 10 15

Pro Pro Ser Ala Ser Ala Tyr Val Lys Leu Val Leu Leu Gly Leu Ile
20 25 30

Met Cys Val Ser Leu Ala Gly Asn Ala Ile Leu Ser Leu Leu Val Leu
35 40 45

Lys Glu Arg Ala Leu His Lys Ala Pro Tyr Tyr Phe Leu Leu Asp Leu
50 55 60

Cys Leu Ala Asp Gly Ile Arg Ser Ala Val Cys Phe Pro Phe Val Leu
65 70 75 80

Ala Ser Val Arg His Gly Ser Ser Trp Thr Phe Ser Ala Leu Ser Cys
85 90 95

Lys Ile Val Ala Phe Met Ala Val Leu Phe Cys Phe His Ala Ala Phe
100 105 110

Met Leu Phe Cys Ile Ser Val Thr Arg Tyr Met Ala Ile Ala His His
115 120 125

Arg Phe Tyr Ala Lys Arg Met Thr Leu Trp Thr Cys Ala Ala Val Ile
130 135 140

Cys Met Ala Trp Thr Leu Ser Val Ala Met Ala Phe Pro Pro Val Phe
145 150 155 160

Asp Val Gly Thr Tyr Lys Phe Ile Arg Glu Glu Asp Gln Cys Ile Phe
165 170 175

Glu His Arg Tyr Phe Lys Ala Asn Asp Thr Leu Gly Phe Met Leu Met
180 185 190

Leu Ala Val Leu Met Ala Ala Thr His Ala Val Tyr Gly Lys Leu Leu
195 200 205

Leu Phe Glu Tyr Arg His Arg Lys Met Lys Pro Val Gln Met Val Pro
210 215 220

Ala Ile Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Thr Gly Gln
225 230 235 240

Ala Ala Ala Asn Trp Ile Ala Gly Phe Gly Arg Gly Pro Met Pro Pro
245 250 255

Thr Leu Leu Gly Ile Arg Gln Asn Gly His Ala Ala Ser Arg Arg Leu
260 265 270

Leu Gly Met Asp Glu Val Lys Gly Glu Lys Gln Leu Gly Arg Met Phe
275 280 285

Tyr Ala Ile Thr Leu Leu Phe Leu Leu Leu Trp Ser Pro Tyr Ile Val
290 295 300

Ala Cys Tyr Trp Arg Val Phe Val Lys Ala Cys Ala Val Pro His Arg
305 310 315 320

Tyr Leu Ala Thr Ala Val Trp Met Ser Phe Ala Gln Ala Ala Val Asn
325 330 335

Pro Ile Val Cys Phe Leu Leu Asn Lys Asp Leu Lys Lys Cys Leu Arg
340 345 350

Thr His Ala Pro Cys Trp Gly Thr Gly Gly Ala Pro Ala Pro Arg Glu
355 360 365

Pro Tyr Cys Val Met
370

<210> 7
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Forward primer

<400> 7
aaaatctaga cgcgatggcg aacgcgagcg a 31

<210> 8
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 8
aaaatctaga gtctatgtgg cggggcctcc c 31

<210> 9
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Forward primer

<400> 9
aaaatctaga tctatggcga actatagcca tgca 34

<210> 10

<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 10
aaaatctaga aaggctaaag atttacagat gctcc 35

<210> 11
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Forward primer

<400> 11
aaaatctaga gtatggccaa cactaccgga gag 33

<210> 12
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 12
aaaatctaga cctgtctgcc taccagcctg c 31

<210> 13
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:FLAG epitope

<400> 13
atggactaca aggacgacga tgacaagggg atcctg 36

<210> 14
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:FLAG epitope

<400> 14

Met Asp Tyr Lys Asp Asp Asp Asp Lys Gly Ile Leu
1 5 10

<210> 15
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Forward primer

<400> 15
aaaatctaga cggcgatggc gaacgctagt ga 32

<210> 16
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 16
aaaatctaga cactttgaga gtcttgtgaa ggc 33

<210> 17
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Forward primer

<400> 17
aaaatctaga tctatggcga actatagcca tgc 33

<210> 18
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Forward primer

<400> 18
aaaatctaga aaggctaaag atttacagat gctcc 35

<210> 19
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 19
aaaatctaga caaatactga actggccgat cccc 34

<210> 20
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:reverse primer

<400> 20
aaaatctaga tgttggcccc agtatggtga tcat 34

<210> 21
<211> 1134
<212> DNA
<213> Rattus sp.

<220>
<221> CDS
<222> (1)..(1131)
<223> Rat SREB1

<400> 21
atg gcg aac gct agt gag ccg ggc ggc ggc ggc ggc ggg gcc gag gct 48
Met Ala Asn Ala Ser Glu Pro Gly Gly Gly Gly Gly Gly Ala Glu Ala
1 5 10 15

gcc gcg ctg ggc ctc agg ctg gcc aca ctc agc ctg ctg ctg tgc gtg 96
Ala Ala Leu Gly Leu Arg Leu Ala Thr Leu Ser Leu Leu Leu Cys Val
20 25 30

agc ctg gcg ggc aac gtg ctg ttc gct ctg ctc atc gtg agg gag cgc 144
Ser Leu Ala Gly Asn Val Leu Phe Ala Leu Leu Ile Val Arg Glu Arg
35 40 45

agc ctg cac cgc gcg cct tac tac ctg ctg ctc gac ctg tgc ctg gcc 192
Ser Leu His Arg Ala Pro Tyr Tyr Leu Leu Leu Asp Leu Cys Leu Ala
50 55 60

gac ggg ctg cgc gcg ctc gcc tgt ctc ccg gcc gtc atg ctg gct gcg 240
Asp Gly Leu Arg Ala Leu Ala Cys Leu Pro Ala Val Met Leu Ala Ala
65 70 75 80

cgg cgc gcg gca gcc gcg gcg ggg acg cct ccg ggt gcg ctg ggc tgc 288
Arg Arg Ala Ala Ala Ala Ala Gly Thr Pro Pro Gly Ala Leu Gly Cys
85 90 95

aag ctg ctg gcc ttc ctg gcc gcg ctc ttc tgc ttc cac gcg gcc ttc 336

```
Lys Leu Leu Ala Phe Leu Ala Ala Leu Phe Cys Phe His Ala Ala Phe
                100                 105                 110

ctg ctg ctg ggc gtg ggc gtc acc cgc tac ctg gcc atc gct cac cac   384
Leu Leu Leu Gly Val Gly Val Thr Arg Tyr Leu Ala Ile Ala His His
        115                 120                 125

cgc ttc tat gcc gag cgc ctg gcc ggc tgg ccg tgc gcc gcg atg ctg   432
Arg Phe Tyr Ala Glu Arg Leu Ala Gly Trp Pro Cys Ala Ala Met Leu
    130                 135                 140

gtg tgc gcc gcc tgg gcg ctg gct ttg gcc gcg gcc ttc ccg ccg gtg   480
Val Cys Ala Ala Trp Ala Leu Ala Leu Ala Ala Ala Phe Pro Pro Val
145                 150                 155                 160

ctg gac ggc ggt ggc gcg gac gac gag gat gcg ccg tgc gcc ctg gag   528
Leu Asp Gly Gly Gly Ala Asp Asp Glu Asp Ala Pro Cys Ala Leu Glu
                165                 170                 175

cag cgg ccc gac ggc gcc ccg ggt gcg cta ggc ttc ctg ctg ctc ctg   576
Gln Arg Pro Asp Gly Ala Pro Gly Ala Leu Gly Phe Leu Leu Leu Leu
            180                 185                 190

gcc gcg gtg gtg ggc gcc acg cac ctc gtc tac ctt cgc ctg ctc ttc   624
Ala Ala Val Val Gly Ala Thr His Leu Val Tyr Leu Arg Leu Leu Phe
        195                 200                 205

ttc atc cac gac cgc cgc aag atg cgg ccc gca cgc ctg gtg ccc gcc   672
Phe Ile His Asp Arg Arg Lys Met Arg Pro Ala Arg Leu Val Pro Ala
    210                 215                 220

gtc agc cac gac tgg acc ttc cac ggc ccg ggc gcc acc ggt caa gcg   720
Val Ser His Asp Trp Thr Phe His Gly Pro Gly Ala Thr Gly Gln Ala
225                 230                 235                 240

gcc gcc aac tgg acg gcg ggc ttc ggc cgc ggg ccc acg cca cct gcg   768
Ala Ala Asn Trp Thr Ala Gly Phe Gly Arg Gly Pro Thr Pro Pro Ala
                245                 250                 255

ctc gtg ggc atc agg cct gca ggc ccg ggc cgc gga gcc cgg cgc ctc   816
Leu Val Gly Ile Arg Pro Ala Gly Pro Gly Arg Gly Ala Arg Arg Leu
            260                 265                 270

ctg gtg ctg gag gaa ttc aag acg gag aag agg ctg tgc aag atg ttc   864
Leu Val Leu Glu Glu Phe Lys Thr Glu Lys Arg Leu Cys Lys Met Phe
        275                 280                 285

tac gcc atc acg ctg ctc ttc ctg ctc ctc tgg ggg ccc tat gtg gtt   912
Tyr Ala Ile Thr Leu Leu Phe Leu Leu Leu Trp Gly Pro Tyr Val Val
    290                 295                 300

gcc agt tac ctg cgc gtc ctg gtg cgg ccc gga gct gtc ccg cag gcc   960
Ala Ser Tyr Leu Arg Val Leu Val Arg Pro Gly Ala Val Pro Gln Ala
305                 310                 315                 320

tac ctg aca gcc tcg gtg tgg ctg aca ttc gca cag gcc ggc atc aac   1008
```

```
Tyr Leu Thr Ala Ser Val Trp Leu Thr Phe Ala Gln Ala Gly Ile Asn
                325                 330                 335

ccc gtg gtg tgt ttc ctc ttc aac cgg gag ctg agg gac tgt ttc aga   1056
Pro Val Val Cys Phe Leu Phe Asn Arg Glu Leu Arg Asp Cys Phe Arg
            340                 345                 350

gcc cag ttc ccc tgt tgc cag agc ccc cag gcc acg cag gcc acc ctc   1104
Ala Gln Phe Pro Cys Cys Gln Ser Pro Gln Ala Thr Gln Ala Thr Leu
        355                 360                 365

ccc tgc gac ctg aaa ggc att ggt ttg tga                           1134
Pro Cys Asp Leu Lys Gly Ile Gly Leu
    370                 375
```

<210> 22
<211> 377
<212> PRT
<213> Rattus sp.

<400> 22

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Met Ala Asn Ala Ser Glu Pro Gly Gly Gly Gly Gly Gly Ala Glu Ala
  1               5                  10                  15

Ala Ala Leu Gly Leu Arg Leu Ala Thr Leu Ser Leu Leu Leu Cys Val
             20                  25                  30

Ser Leu Ala Gly Asn Val Leu Phe Ala Leu Leu Ile Val Arg Glu Arg
         35                  40                  45

Ser Leu His Arg Ala Pro Tyr Tyr Leu Leu Leu Asp Leu Cys Leu Ala
     50                  55                  60

Asp Gly Leu Arg Ala Leu Ala Cys Leu Pro Ala Val Met Leu Ala Ala
 65                  70                  75                  80

Arg Arg Ala Ala Ala Ala Ala Gly Thr Pro Pro Gly Ala Leu Gly Cys
                 85                  90                  95

Lys Leu Leu Ala Phe Leu Ala Ala Leu Phe Cys Phe His Ala Ala Phe
            100                 105                 110

Leu Leu Leu Gly Val Gly Val Thr Arg Tyr Leu Ala Ile Ala His His
        115                 120                 125

Arg Phe Tyr Ala Glu Arg Leu Ala Gly Trp Pro Cys Ala Ala Met Leu
    130                 135                 140

Val Cys Ala Ala Trp Ala Leu Ala Leu Ala Ala Ala Phe Pro Pro Val
145                 150                 155                 160

Leu Asp Gly Gly Gly Ala Asp Asp Glu Asp Ala Pro Cys Ala Leu Glu
                165                 170                 175

Gln Arg Pro Asp Gly Ala Pro Gly Ala Leu Gly Phe Leu Leu Leu Leu
```

180 185 190

Ala Ala Val Val Gly Ala Thr His Leu Val Tyr Leu Arg Leu Leu Phe
195 200 205

Phe Ile His Asp Arg Arg Lys Met Arg Pro Ala Arg Leu Val Pro Ala
210 215 220

Val Ser His Asp Trp Thr Phe His Gly Pro Gly Ala Thr Gly Gln Ala
225 230 235 240

Ala Ala Asn Trp Thr Ala Gly Phe Gly Arg Gly Pro Thr Pro Pro Ala
245 250 255

Leu Val Gly Ile Arg Pro Ala Gly Pro Gly Arg Gly Ala Arg Arg Leu
260 265 270

Leu Val Leu Glu Glu Phe Lys Thr Glu Lys Arg Leu Cys Lys Met Phe
275 280 285

Tyr Ala Ile Thr Leu Leu Phe Leu Leu Leu Trp Gly Pro Tyr Val Val
290 295 300

Ala Ser Tyr Leu Arg Val Leu Val Arg Pro Gly Ala Val Pro Gln Ala
305 310 315 320

Tyr Leu Thr Ala Ser Val Trp Leu Thr Phe Ala Gln Ala Gly Ile Asn
325 330 335

Pro Val Val Cys Phe Leu Phe Asn Arg Glu Leu Arg Asp Cys Phe Arg
340 345 350

Ala Gln Phe Pro Cys Cys Gln Ser Pro Gln Ala Thr Gln Ala Thr Leu
355 360 365

Pro Cys Asp Leu Lys Gly Ile Gly Leu
370 375

<210> 23
<211> 1113
<212> DNA
<213> Rattus sp.

<220>
<221> CDS
<222> (1)..(1110)
<223> Rat SREB2

<400> 23
atg gcg aac tat agc cat gca gct gac aac att ttg caa aat ctc tcg 48
Met Ala Asn Tyr Ser His Ala Ala Asp Asn Ile Leu Gln Asn Leu Ser
1 5 10 15

cct cta aca gcc ttt ctg aaa ctg act tcc ttg ggt ttc ata ata gga 96
Pro Leu Thr Ala Phe Leu Lys Leu Thr Ser Leu Gly Phe Ile Ile Gly

```
                  20                  25                  30

gtc agt gtg gtg ggc aac ctt ctg atc tcc att ttg cta gtg aaa gat   144
Val Ser Val Val Gly Asn Leu Leu Ile Ser Ile Leu Leu Val Lys Asp
          35                  40                  45

aag acc ttg cat aga gct cct tac tac ttc ctg ctg gat ctg tgc tgc   192
Lys Thr Leu His Arg Ala Pro Tyr Tyr Phe Leu Leu Asp Leu Cys Cys
      50                  55                  60

tca gac atc ctc aga tct gca att tgt ttt cca ttt gta ttc aac tct   240
Ser Asp Ile Leu Arg Ser Ala Ile Cys Phe Pro Phe Val Phe Asn Ser
 65                  70                  75                  80

gtc aaa aat ggc tct acc tgg act tac ggg act ctg act tgc aaa gtg   288
Val Lys Asn Gly Ser Thr Trp Thr Tyr Gly Thr Leu Thr Cys Lys Val
                 85                  90                  95

att gcc ttt ctg ggg gtt ttg tcc tgt ttc cac act gcc ttc atg ctc   336
Ile Ala Phe Leu Gly Val Leu Ser Cys Phe His Thr Ala Phe Met Leu
            100                 105                 110

ttc tgc atc agc gtc acc aga tac tta gcc atc gcc cat cac cgc ttc   384
Phe Cys Ile Ser Val Thr Arg Tyr Leu Ala Ile Ala His His Arg Phe
        115                 120                 125

tat aca aag agg ctg acc ttt tgg acg tgt ttg gct gtg atc tgc atg   432
Tyr Thr Lys Arg Leu Thr Phe Trp Thr Cys Leu Ala Val Ile Cys Met
    130                 135                 140

gtg tgg act ctg tct gtg gcc atg gca ttt ccc cca gtt tta gat gta   480
Val Trp Thr Leu Ser Val Ala Met Ala Phe Pro Pro Val Leu Asp Val
145                 150                 155                 160

ggc acc tac tca ttc att agg gag gag gat cag tgt acc ttc caa cac   528
Gly Thr Tyr Ser Phe Ile Arg Glu Glu Asp Gln Cys Thr Phe Gln His
                165                 170                 175

cgc tcc ttc agg gct aac gat tcc cta gga ttt atg ctg ctc ctt gct   576
Arg Ser Phe Arg Ala Asn Asp Ser Leu Gly Phe Met Leu Leu Leu Ala
            180                 185                 190

ctc atc ctc cta gcc aca cag ctt gtc tac ctc aag ctg ata ttt ttt   624
Leu Ile Leu Leu Ala Thr Gln Leu Val Tyr Leu Lys Leu Ile Phe Phe
        195                 200                 205

gtc cac gat cga agg aaa atg aag cca gtc cag ttt gta gca gca gtg   672
Val His Asp Arg Arg Lys Met Lys Pro Val Gln Phe Val Ala Ala Val
    210                 215                 220

agt cag aac tgg acc ttt cat ggc cct gga gct agt ggc cag gca gct   720
Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Ser Gly Gln Ala Ala
225                 230                 235                 240

gcc aat tgg cta gca gga ttt gga agg ggt ccc aca cca ccc acc ttg   768
Ala Asn Trp Leu Ala Gly Phe Gly Arg Gly Pro Thr Pro Pro Thr Leu
```

```
                    245                 250                 255

ctg ggc atc agg caa aat gcg aat acc aca ggc aga aga cgg ctc ttg   816
Leu Gly Ile Arg Gln Asn Ala Asn Thr Thr Gly Arg Arg Arg Leu Leu
            260                 265                 270

gtt ttg gat gag ttc aaa atg gag aaa aga atc agc aga atg ttc tat   864
Val Leu Asp Glu Phe Lys Met Glu Lys Arg Ile Ser Arg Met Phe Tyr
        275                 280                 285

ata atg act ttc ctc ttc cta acc ttg tgg ggt ccc tac ctg gtg gcc   912
Ile Met Thr Phe Leu Phe Leu Thr Leu Trp Gly Pro Tyr Leu Val Ala
    290                 295                 300

tgc tat tgg aga gtt ttt gca aga ggg cct gta gta cca ggg gga ttt   960
Cys Tyr Trp Arg Val Phe Ala Arg Gly Pro Val Val Pro Gly Gly Phe
305                 310                 315                 320

cta aca gcc gct gtc tgg atg agt ttc gcc caa gca gga atc aat ccc  1008
Leu Thr Ala Ala Val Trp Met Ser Phe Ala Gln Ala Gly Ile Asn Pro
                325                 330                 335

ttt gtc tgc att ttc tcc aac agg gag ctg agg cgc tgt ttc agc aca  1056
Phe Val Cys Ile Phe Ser Asn Arg Glu Leu Arg Arg Cys Phe Ser Thr
            340                 345                 350

acc ctt ctt tac tgc aga aaa tcc agg tta cca agg gaa cct tac tgt  1104
Thr Leu Leu Tyr Cys Arg Lys Ser Arg Leu Pro Arg Glu Pro Tyr Cys
        355                 360                 365

gtt ata tga                                                     1113
Val Ile
    370
```

<210> 24
<211> 370
<212> PRT
<213> Rattus sp.

<400> 24

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Met Ala Asn Tyr Ser His Ala Ala Asp Asn Ile Leu Gln Asn Leu Ser
  1               5                  10                  15

Pro Leu Thr Ala Phe Leu Lys Leu Thr Ser Leu Gly Phe Ile Ile Gly
            20                  25                  30

Val Ser Val Val Gly Asn Leu Leu Ile Ser Ile Leu Leu Val Lys Asp
        35                  40                  45

Lys Thr Leu His Arg Ala Pro Tyr Tyr Phe Leu Leu Asp Leu Cys Cys
    50                  55                  60

Ser Asp Ile Leu Arg Ser Ala Ile Cys Phe Pro Phe Val Phe Asn Ser
65                  70                  75                  80
```

Val Lys Asn Gly Ser Thr Trp Thr Tyr Gly Thr Leu Thr Cys Lys Val
85 90 95

Ile Ala Phe Leu Gly Val Leu Ser Cys Phe His Thr Ala Phe Met Leu
100 105 110

Phe Cys Ile Ser Val Thr Arg Tyr Leu Ala Ile Ala His His Arg Phe
115 120 125

Tyr Thr Lys Arg Leu Thr Phe Trp Thr Cys Leu Ala Val Ile Cys Met
130 135 140

Val Trp Thr Leu Ser Val Ala Met Ala Phe Pro Pro Val Leu Asp Val
145 150 155 160

Gly Thr Tyr Ser Phe Ile Arg Glu Glu Asp Gln Cys Thr Phe Gln His
165 170 175

Arg Ser Phe Arg Ala Asn Asp Ser Leu Gly Phe Met Leu Leu Leu Ala
180 185 190

Leu Ile Leu Leu Ala Thr Gln Leu Val Tyr Leu Lys Leu Ile Phe Phe
195 200 205

Val His Asp Arg Arg Lys Met Lys Pro Val Gln Phe Val Ala Ala Val
210 215 220

Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Ser Gly Gln Ala Ala
225 230 235 240

Ala Asn Trp Leu Ala Gly Phe Gly Arg Gly Pro Thr Pro Pro Thr Leu
245 250 255

Leu Gly Ile Arg Gln Asn Ala Asn Thr Thr Gly Arg Arg Arg Leu Leu
260 265 270

Val Leu Asp Glu Phe Lys Met Glu Lys Arg Ile Ser Arg Met Phe Tyr
275 280 285

Ile Met Thr Phe Leu Phe Leu Thr Leu Trp Gly Pro Tyr Leu Val Ala
290 295 300

Cys Tyr Trp Arg Val Phe Ala Arg Gly Pro Val Val Pro Gly Gly Phe
305 310 315 320

Leu Thr Ala Ala Val Trp Met Ser Phe Ala Gln Ala Gly Ile Asn Pro
325 330 335

Phe Val Cys Ile Phe Ser Asn Arg Glu Leu Arg Arg Cys Phe Ser Thr
340 345 350

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Val Ile
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ctg cca tca gca tcg gct tat gtg aag ctg gtg ctg ctg gga ctg atc   96
Leu Pro Ser Ala Ser Ala Tyr Val Lys Leu Val Leu Leu Gly Leu Ile
            20                  25                  30

atg tgt gta agc ctg gca ggc aat gcc atc ttg tcc ctg ctg gtg ctc  144
Met Cys Val Ser Leu Ala Gly Asn Ala Ile Leu Ser Leu Leu Val Leu
        35                  40                  45

aag gag cgt gcc ctg cac aag gct cct tac tac ttt ctg ctg gac ctg  192
Lys Glu Arg Ala Leu His Lys Ala Pro Tyr Tyr Phe Leu Leu Asp Leu
    50                  55                  60

tgc cta gcc gat ggc ata cgc tct gcc atc tgc ttc ccc ttt gta ctg  240
Cys Leu Ala Asp Gly Ile Arg Ser Ala Ile Cys Phe Pro Phe Val Leu
65                  70                  75                  80

gct tct gtg cgc cat ggc tcc tcg tgg acc ttc agt gca ctc agc tgt  288
Ala Ser Val Arg His Gly Ser Ser Trp Thr Phe Ser Ala Leu Ser Cys
                85                  90                  95

aag att gtg gcc ttt atg gct gtg ctc ttt tgc ttc cat gcg gcc ttc  336
Lys Ile Val Ala Phe Met Ala Val Leu Phe Cys Phe His Ala Ala Phe
            100                 105                 110

atg ctg ttc tgc atc agc gtc acc cgc tac atg gcc atc gcc cac cac  384
Met Leu Phe Cys Ile Ser Val Thr Arg Tyr Met Ala Ile Ala His His
        115                 120                 125

cgc ttc tat gcc aag cgc atg aca ctc tgg aca tgc gca gct gtc atc  432
Arg Phe Tyr Ala Lys Arg Met Thr Leu Trp Thr Cys Ala Ala Val Ile
    130                 135                 140

tgc atg gcc tgg acc ttg tct gtg gcc atg gct ttc cca cct gtc ttt  480
Cys Met Ala Trp Thr Leu Ser Val Ala Met Ala Phe Pro Pro Val Phe
145                 150                 155                 160

gat gtg ggc acc tac aag ttt atc cga gag gag gac cag tgc atc ttt  528
Asp Val Gly Thr Tyr Lys Phe Ile Arg Glu Glu Asp Gln Cys Ile Phe
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Glu His Arg Tyr Phe Lys Ala Asn Asp Thr Leu Gly Phe Met Leu Met
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ttg gct gtg ctc atg gca gcc aca cat gct gtc tat ggc aag ctg cta   624
Leu Ala Val Leu Met Ala Ala Thr His Ala Val Tyr Gly Lys Leu Leu
        195                 200                 205

ctc ttc gag tat cgt cac cgc aag atg aag cca gtg cag atg gtg ccc   672
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Ala Ile Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Thr Gly Gln
225                 230                 235                 240

gct gct gcc aac tgg atc gct ggc ttt ggc cgt ggg ccc atg cca cca   768
Ala Ala Ala Asn Trp Ile Ala Gly Phe Gly Arg Gly Pro Met Pro Pro
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act ctg ctg ggt atc cgg cag aat ggg cat gca gct agc cgg cgg cta   816
Thr Leu Leu Gly Ile Arg Gln Asn Gly His Ala Ala Ser Arg Arg Leu
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ctg ggc atg gac gag gtc aag ggt gaa aag cag ctg ggc cga atg ttc   864
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tac gcg att aca ctg ctc ttc ctg ctc ctc tgg tca cca tac att gtg   912
Tyr Ala Ile Thr Leu Leu Phe Leu Leu Leu Trp Ser Pro Tyr Ile Val
    290                 295                 300

gcc tgc tac tgg cga gtg ttt gtg aaa gcc tgc gct gtg ccc cac cgc   960
Ala Cys Tyr Trp Arg Val Phe Val Lys Ala Cys Ala Val Pro His Arg
305                 310                 315                 320

tac ctg gcc act gct gtt tgg atg agc ttc gcc cag gct gct gtc aac  1008
Tyr Leu Ala Thr Ala Val Trp Met Ser Phe Ala Gln Ala Ala Val Asn
                325                 330                 335

cca atc gtc tgc ttc ctg ctt aac aag gac ctc aag aag tgc ctg agg  1056
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act cat gcc cct tgc tgg ggc aca gga ggt gcc cca gct ccc aga gaa  1104
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Met Cys Val Ser Leu Ala Gly Asn Ala Ile Leu Ser Leu Leu Val Leu
        35                  40                  45

Lys Glu Arg Ala Leu His Lys Ala Pro Tyr Tyr Phe Leu Leu Asp Leu
    50                  55                  60

Cys Leu Ala Asp Gly Ile Arg Ser Ala Ile Cys Phe Pro Phe Val Leu
65                  70                  75                  80

Ala Ser Val Arg His Gly Ser Ser Trp Thr Phe Ser Ala Leu Ser Cys
                85                  90                  95

Lys Ile Val Ala Phe Met Ala Val Leu Phe Cys Phe His Ala Ala Phe
            100                 105                 110

Met Leu Phe Cys Ile Ser Val Thr Arg Tyr Met Ala Ile Ala His His
        115                 120                 125

Arg Phe Tyr Ala Lys Arg Met Thr Leu Trp Thr Cys Ala Ala Val Ile
    130                 135                 140

Cys Met Ala Trp Thr Leu Ser Val Ala Met Ala Phe Pro Pro Val Phe
145                 150                 155                 160

Asp Val Gly Thr Tyr Lys Phe Ile Arg Glu Glu Asp Gln Cys Ile Phe
                165                 170                 175

Glu His Arg Tyr Phe Lys Ala Asn Asp Thr Leu Gly Phe Met Leu Met
            180                 185                 190

Leu Ala Val Leu Met Ala Ala Thr His Ala Val Tyr Gly Lys Leu Leu
        195                 200                 205

Leu Phe Glu Tyr Arg His Arg Lys Met Lys Pro Val Gln Met Val Pro
    210                 215                 220

Ala Ile Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Thr Gly Gln
225                 230                 235                 240

Ala Ala Ala Asn Trp Ile Ala Gly Phe Gly Arg Gly Pro Met Pro Pro
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Thr Leu Leu Gly Ile Arg Gln Asn Gly His Ala Ala Ser Arg Arg Leu
            260                 265                 270

Leu Gly Met Asp Glu Val Lys Gly Glu Lys Gln Leu Gly Arg Met Phe
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          275                 280                 285

Tyr Ala Ile Thr Leu Leu Phe Leu Leu Leu Trp Ser Pro Tyr Ile Val
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Ala Cys Tyr Trp Arg Val Phe Val Lys Ala Cys Ala Val Pro His Arg
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Tyr Leu Ala Thr Ala Val Trp Met Ser Phe Ala Gln Ala Ala Val Asn
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Pro Ile Val Cys Phe Leu Leu Asn Lys Asp Leu Lys Lys Cys Leu Arg
            340                 345                 350

Thr His Ala Pro Cys Trp Gly Thr Gly Gly Ala Pro Ala Pro Arg Glu
        355                 360                 365

Pro Tyr Cys Val Met
    370
```

FIG. 1

```
SREB 1  MANASEPGGSGGGEAAALG----LKLATLSLLLCVSLAGN  36
SREB 2  MANYSHAADNILQNLSP--LTAFLKLTSLGFIIGVSVVGN  38
SREB 3  MANTTGEPEEVSGALSPPSASAYVKLVLLGLIMCVSLAGN  40

SREB 1  VLFALLIVRERSLHRAPYYLLLDLCLADGLRALACLPAVM  76
SREB 2  LLISILLVKDKTLHRAPYYFLLDLCCSDILRSAICFPFVF  78
SREB 3  AILSLLVLKERALHKAPYYFLLDLCLADGIRSAVCFPFVL  80

SREB 1  LAARRAAAAAAGAPPGALGCKLLAFLAALFCFHAAFLLLGV 116
SREB 2  NSVKNGSTWTY---GTLTCKVIAFLGVLSCFHTAFMLFCI 115
SREB 3  ASVRHGSSWTF---SALSCKIVAFMAVLFCFHAAFMLFCI 117

SREB 1  GVTRYLAIAHHRFYAERLAGWPCAAMLVCAAWALALAAAF 156
SREB 2  SVTRYLAIAHHRFYTKRLTFWTCLAV-ICMVWTLSVAMAF 154
SREB 3  SVTRYMAIAHHRFYAKRMTLWTCAAV-ICMAWTLSVAMAF 156

SREB 1  PPVLDGGG---DDEDAPCALEQRPDGAPGALGFLLLLAVV 193
SREB 2  PPVLDVGTYSFIREEDQCTFQHRSFRANDSLGFMLLLALI 194
SREB 3  PPVFDVGTYKFIREEDQCIFEHRYFKANDTLGFMLMLAVL 196

SREB 1  VGATHLVYLRLLFFIHDRRKMRPARLVPAVSHDWTFHGPG 233
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SREB 3  MAATHAVYGKLLLFEYRHRKMKPVQMVPAISQNWTFHGPG 236

SREB 1  ATGQAAAANWTAGFGRGPTPPALVGIRPAGPGRGARRLLVL 273
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SREB 3  DEVKGEKQLGRMFYAITLLFLLLWSPYIVACYWRVFVKAC 315

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SREB 2  VVPGGFLTAAVWMSFAQAGINPFVCIFSNRELRRCFSTTL 354
SREB 3  AVPHRYLATAVWMSFAQAAVNPIVCFLLNKDLKKCLRTHA 355

SREB 1  PCCQSPRTTQATHP--CDLKGIGL.                376
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SREB 3  P-CWGTGGAPAPREPYC-----VM.                374
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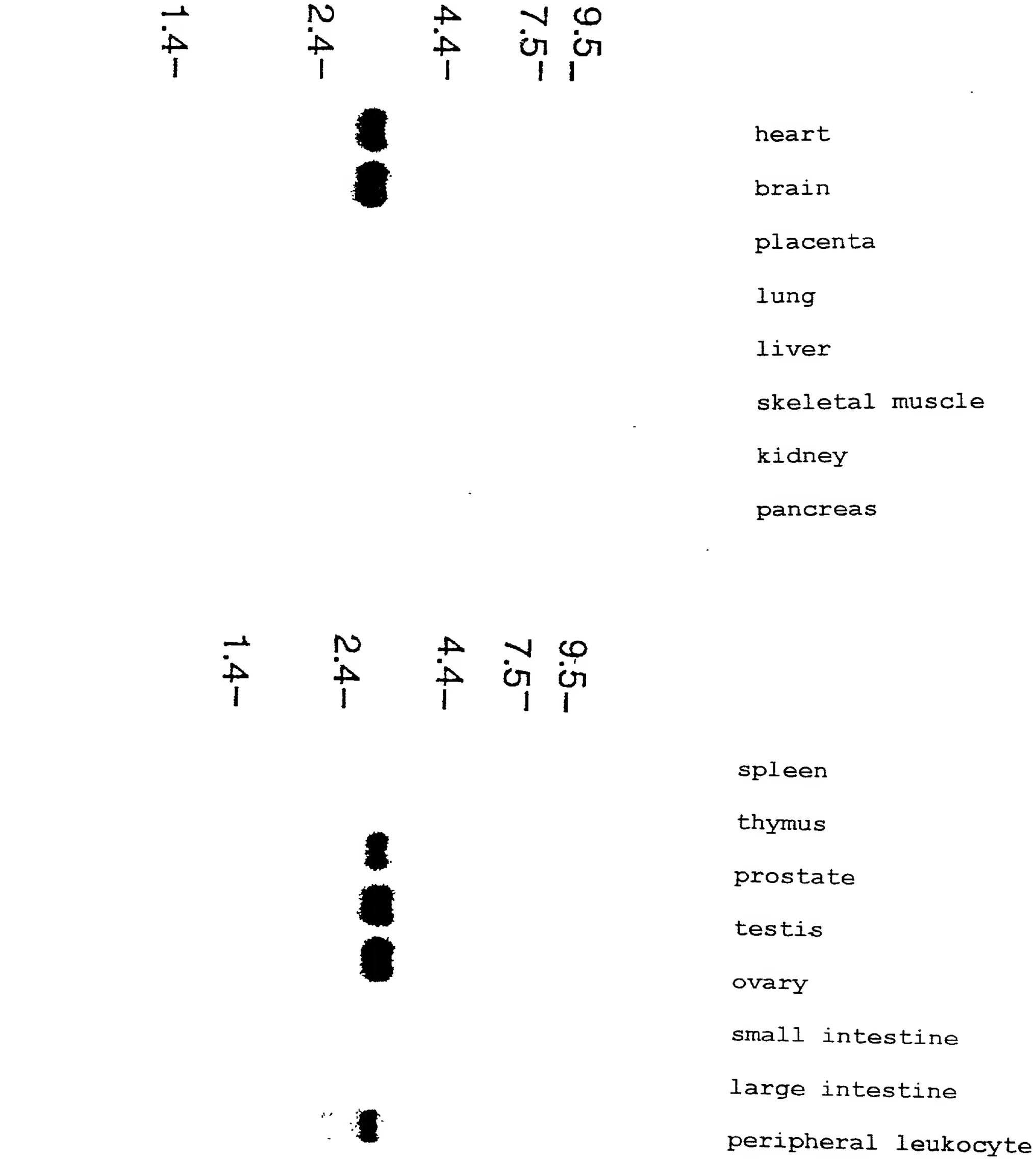
FIG. 2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lung
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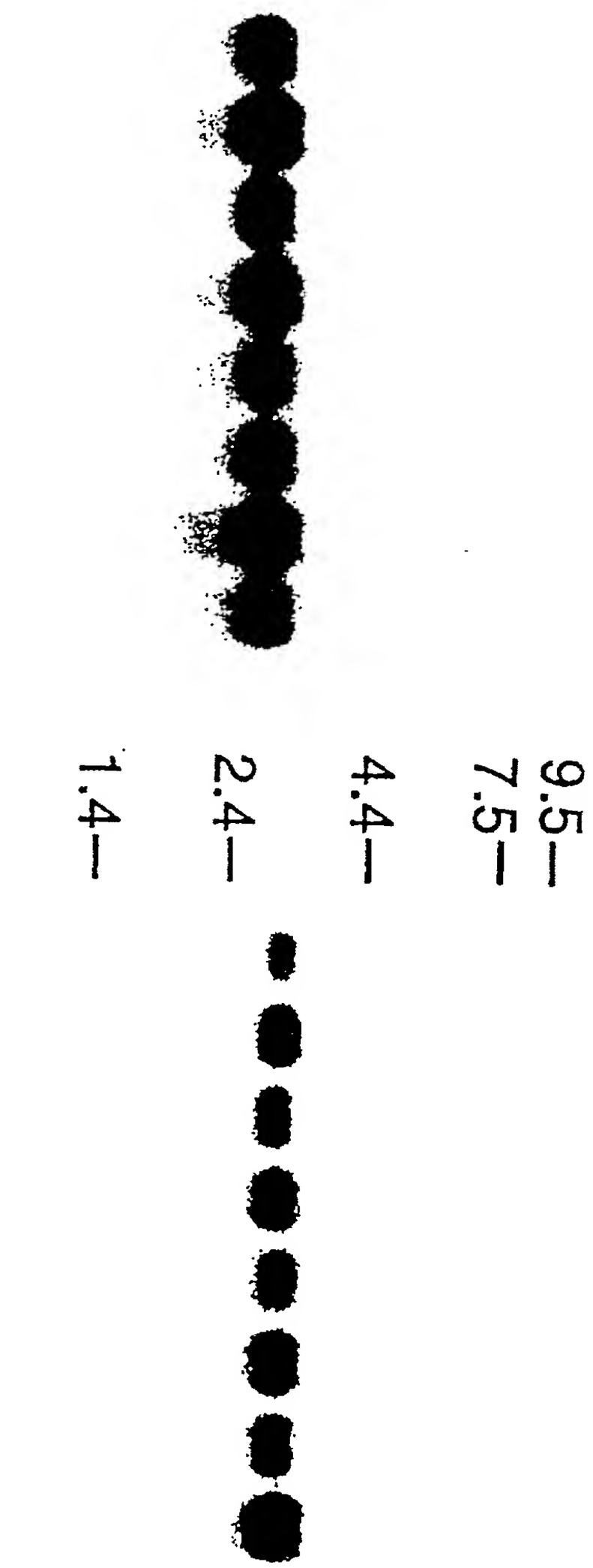
pancreas
spleen
thymus
prostate
testis
ovary
small intestine
large intestine
peripheral leukocyte

FIG. 3



amygdala

caudate nucleus

corpus callosum

hippocampus

whole brain

substania nigra

subthalamic nucleus

thalamus

cerebellum

cerebral cortex

medulla

spinal cord

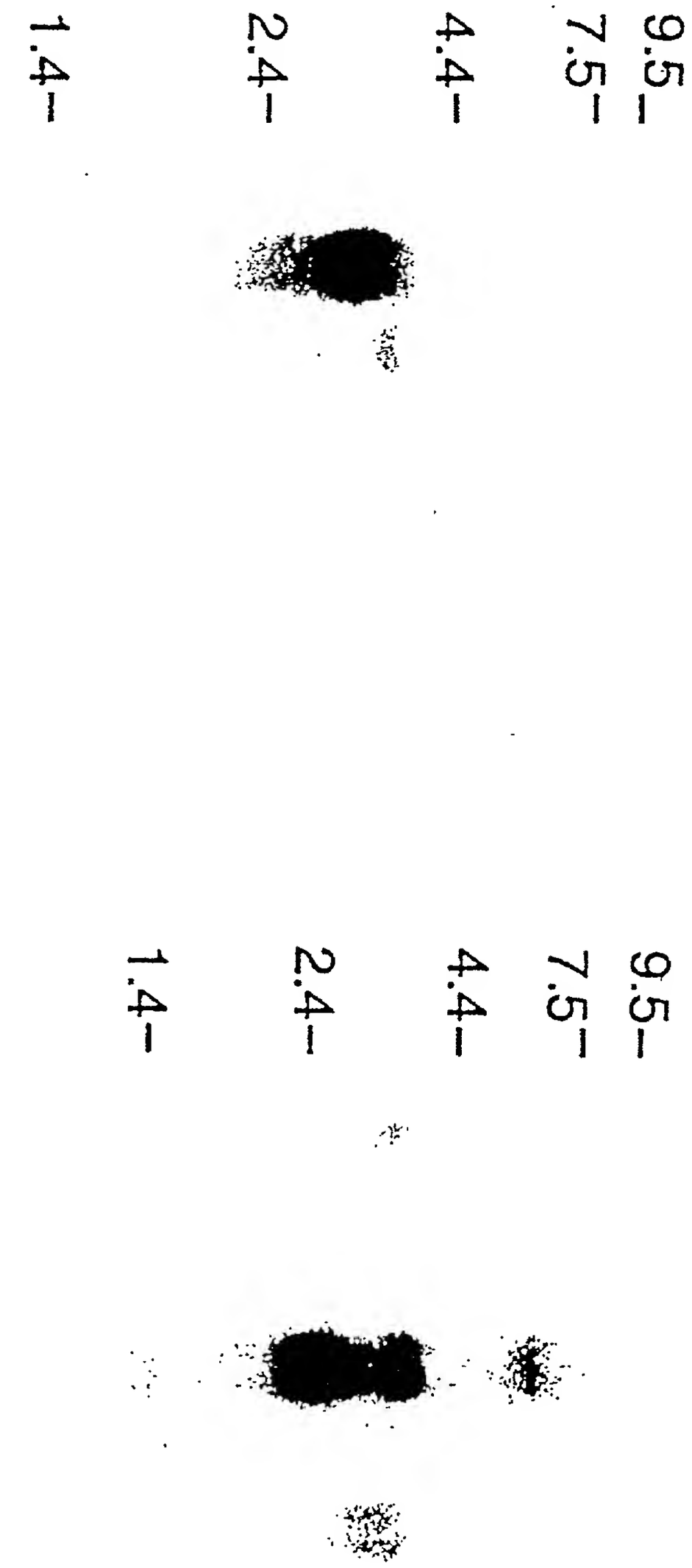
occipital lobe

frontal lobe

temporal lobe

putamen

FIG. 4



heart

brain

placenta

lung

liver

skeletal muscle

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pancreas

spleen

thymus

prostate

testis

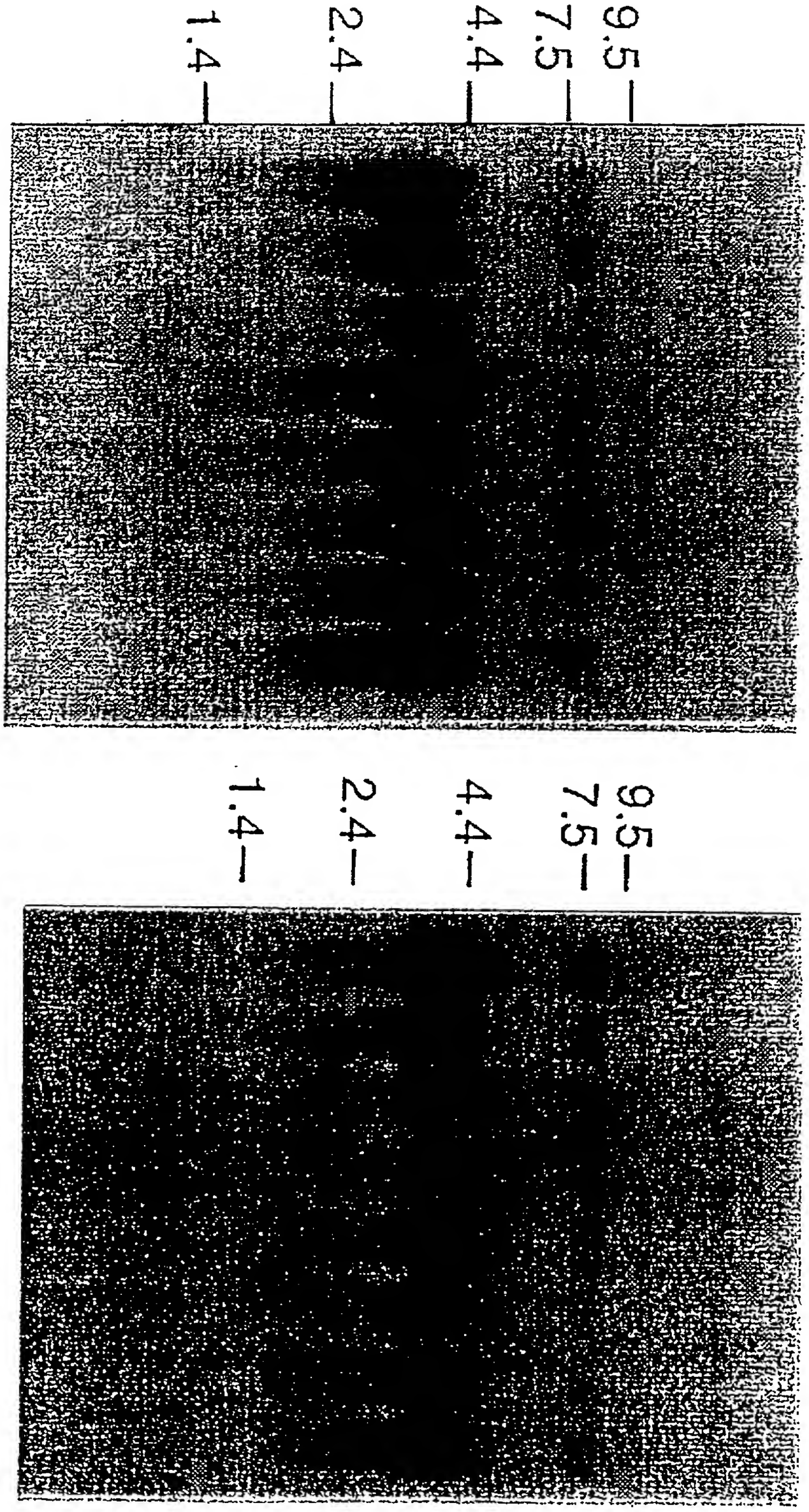
ovary

small intestine

large intestine

peripheral leukocyte

FIG. 5



amygdala

caudate nucleus

corpus callosum

hippocampus

whole brain

substania nigra

subthalamic nucleus

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cerebellum

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medulla

spinal cord

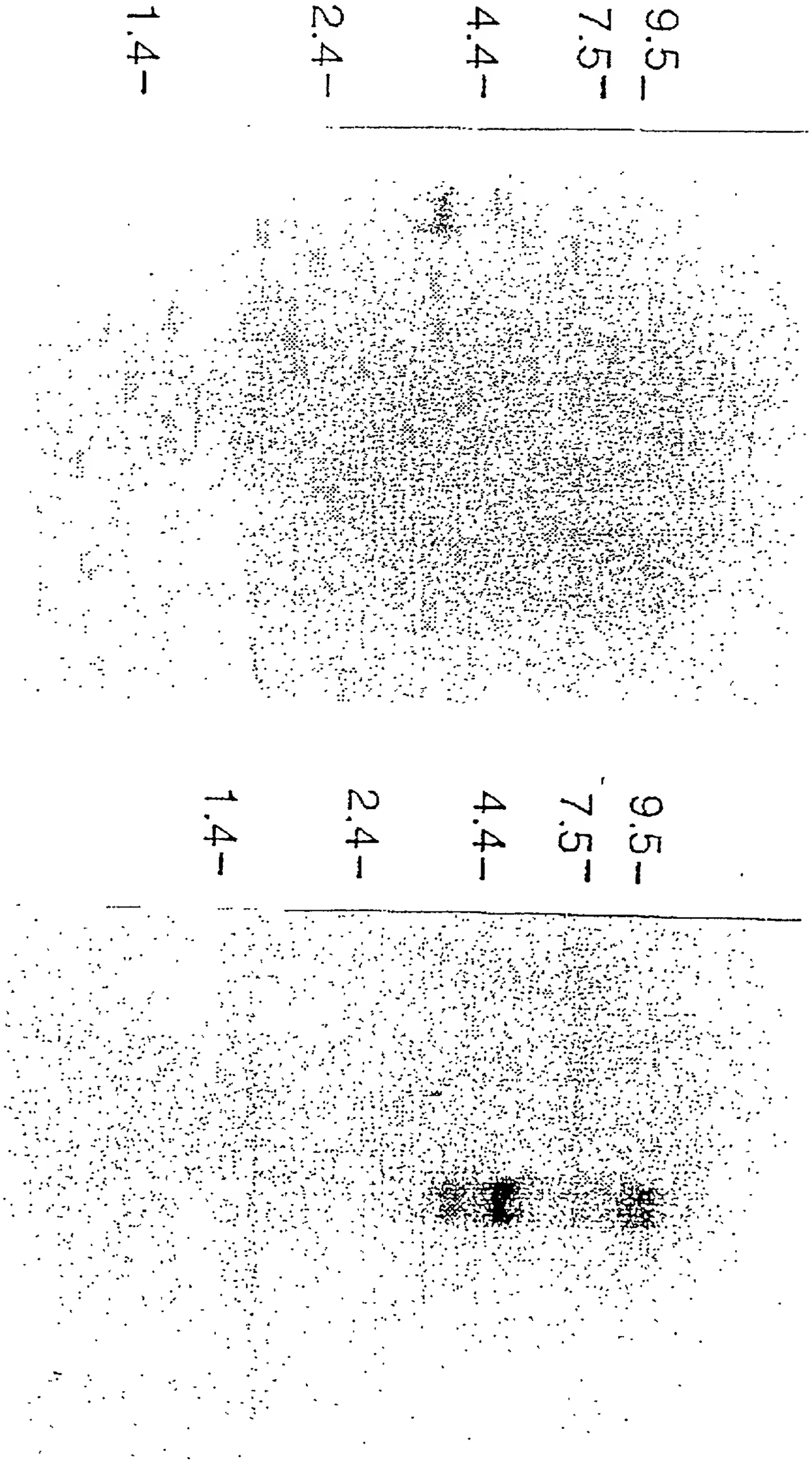
occipital lobe

frontal lobe

temporal lobe

putamen

FIG. 6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ovary
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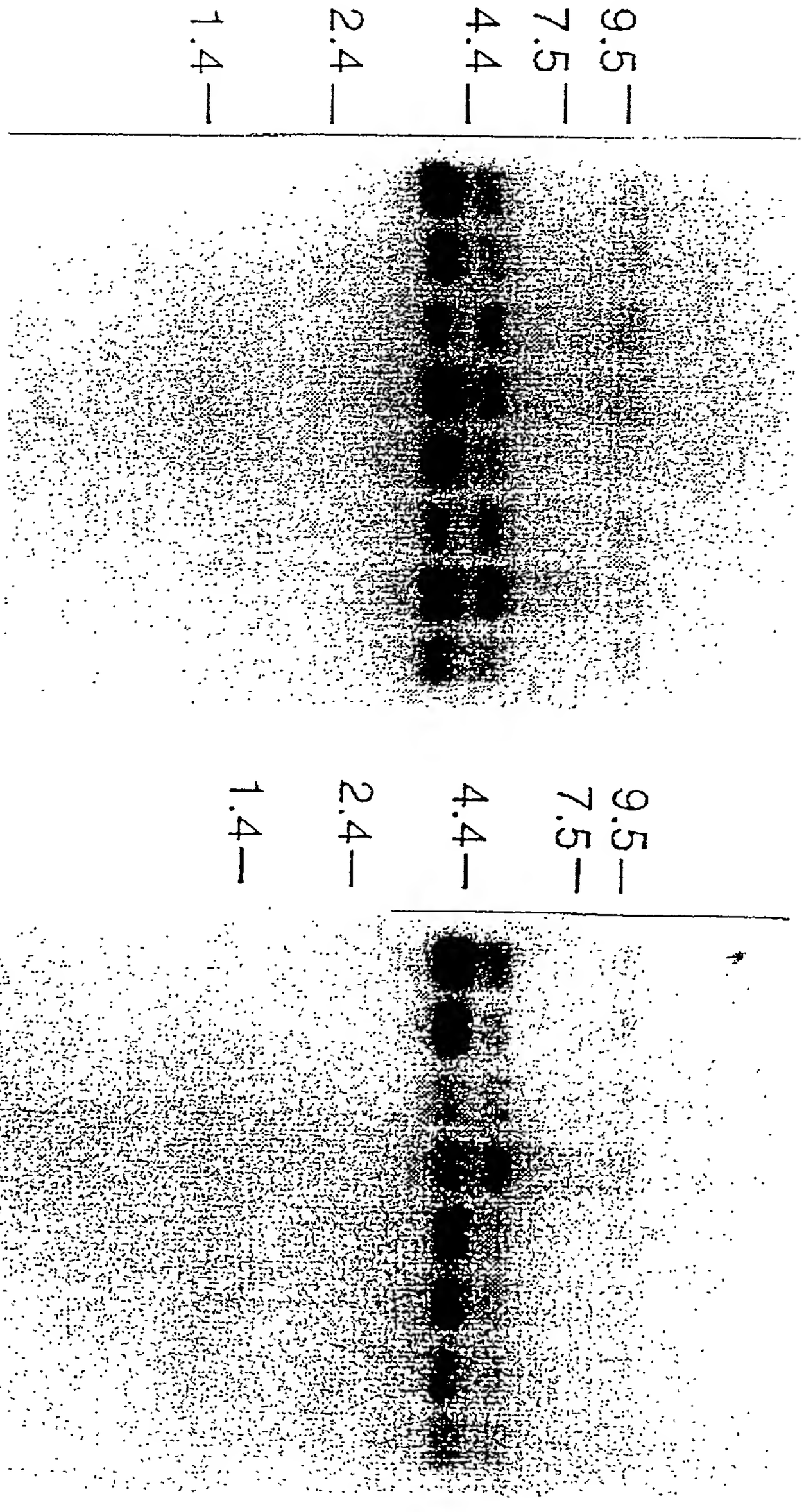
large intestine
peripheral leukocyte

FIG. 7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thalamus
1.4
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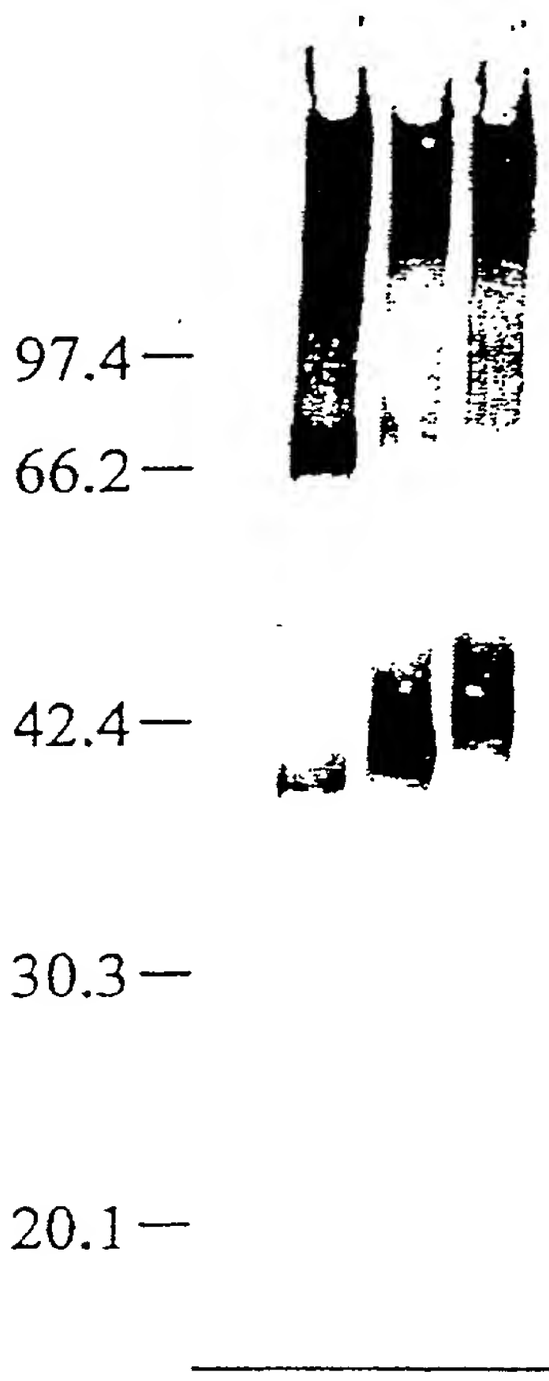
medulla
spinal cord
occipital lobe
frontal lobe
temporal lobe
putamen

FIG. 8



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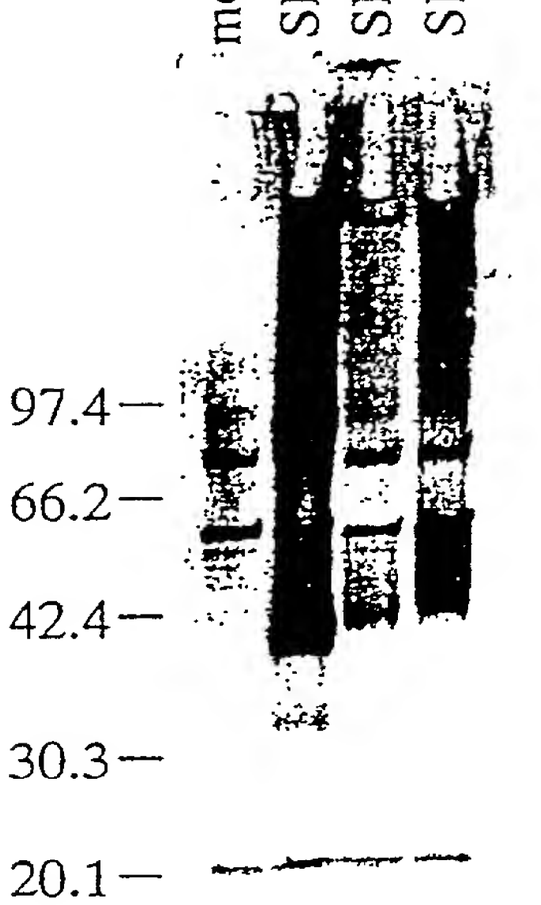
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FIG. 10

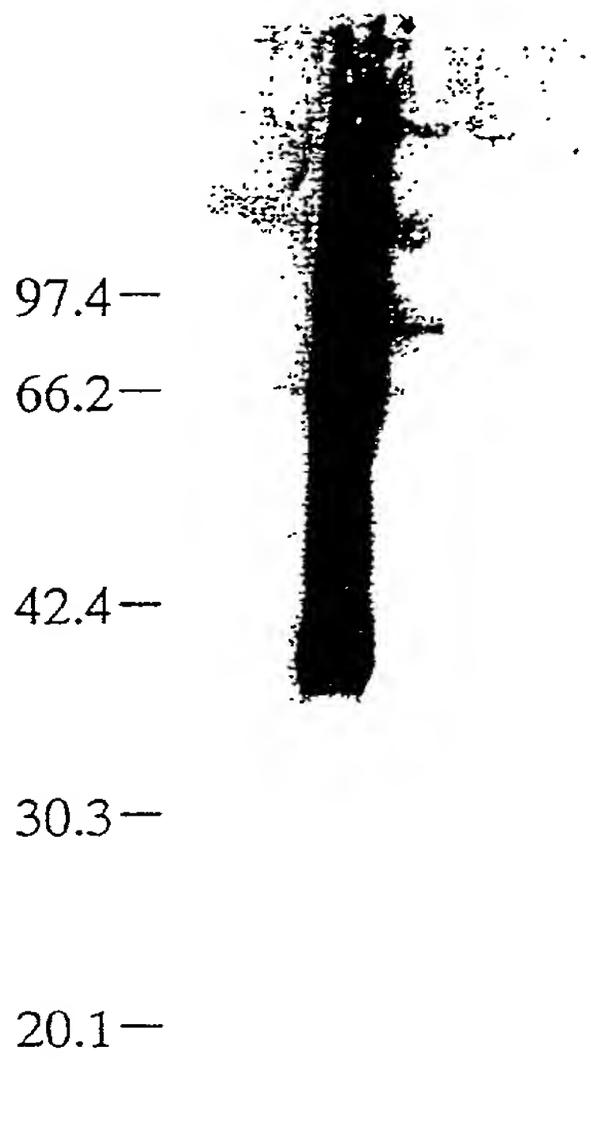
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FIG. 11

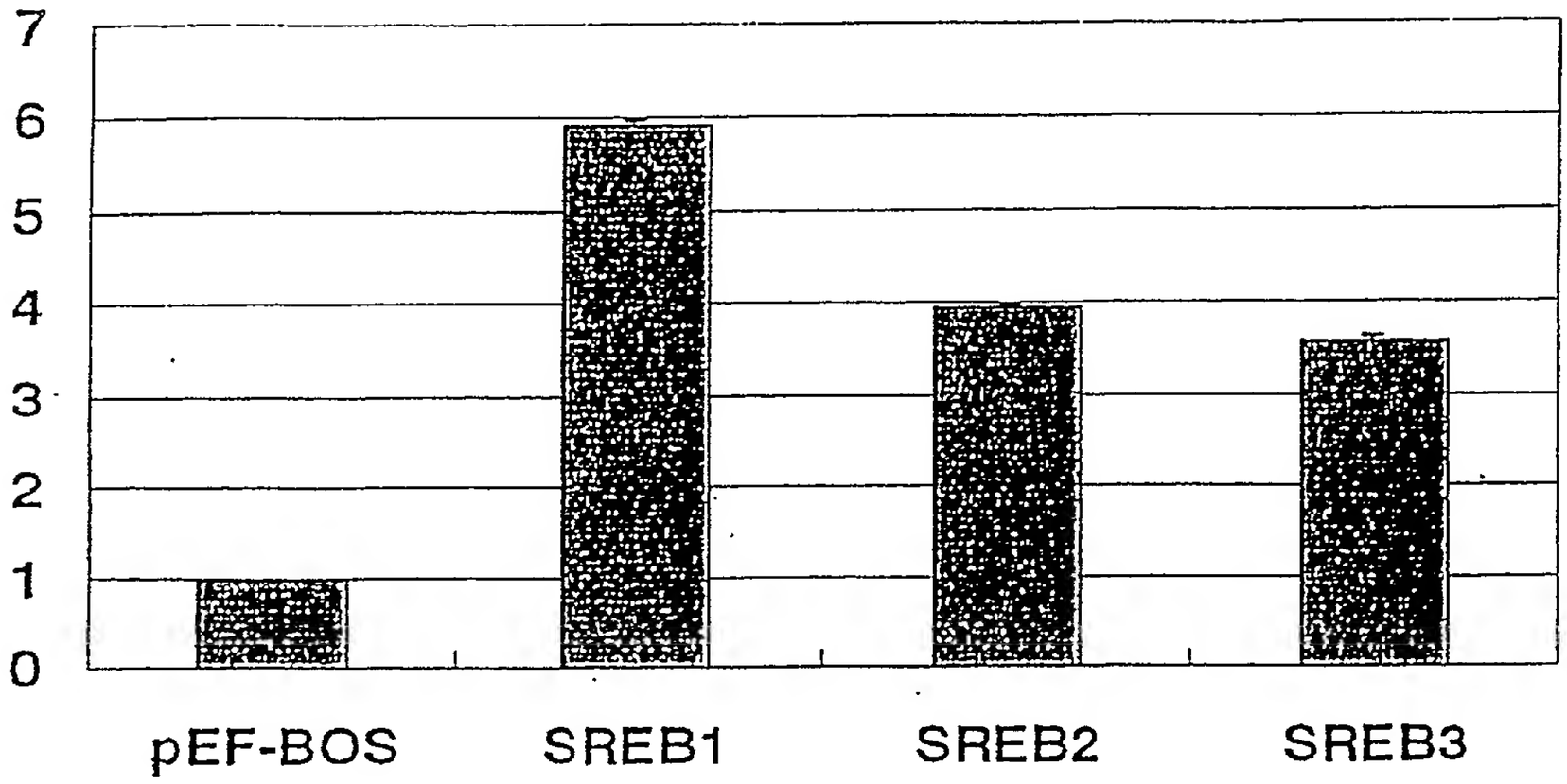
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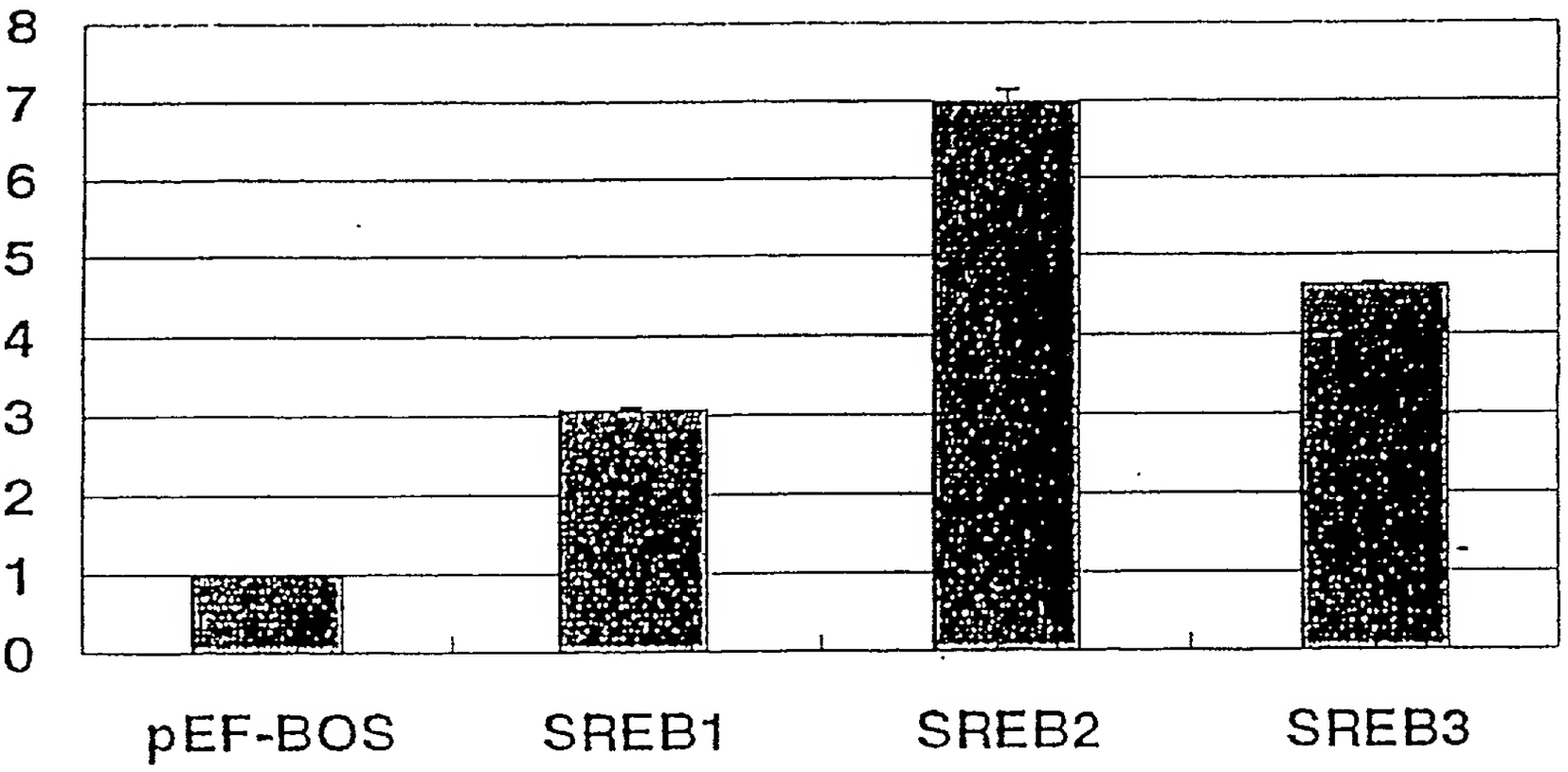
FIG. 12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SREB2
SREB3

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Novel G Protein-coupled Receptor Proteins

the specification of which is attached hereto unless the following box is checked:

☒ was filed on March 11, 1999 as United States Application Number or PCT International Application Number PCT/JP99/01191 and was amended on _____ (if applicable).
I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge the duty to disclose information of which is material to the patentability as defined in 37 CFR § 1.56.
I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
P.Hei.10-060245 (Number)	Japan (Country)	12/March/1998 (Day/Month/Year Filed)	☐
P.Hei.11-026774 (Number)	Japan (Country)	03/February/1999 (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐

I hereby claim the benefits under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)

I hereby claim the benefits under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application Number)	(Filing Date)	(Status – patented, pending, abandoned)
(Application Number)	(Filing Date)	(Status – patented, pending, abandoned)

30 I hereby appoint John H. Mion, Reg. No. 18,879; Thomas J. Macpeak, Reg. No. 19,292; Robert J. Seas, Jr., Reg. No. 21,092; Darryl Mexic, Reg. No. 23,063; Robert V. Sloan, Reg. No. 22,775; Peter D. Olexy, Reg. No. 24,513; J. Frank Osha, Reg. No. 24,625; Waddell A. Biggart, Reg. No. 24,861; Louis Gubinsky, Reg. No. 24,835; Neil B. Siegel, Reg. No. 25,200; David J. Cushing, Reg. No. 28,703; John R. Inge, Reg. No. 26,916; Joseph J. Ruch, Jr., Reg. No. 26,577; Sheldon I. Landsman, Reg. No. 25,430; Richard C. Turner, Reg. No. 29,710; Howard L. Bernstein, Reg. No. 25,665; Alan J. Kasper, Reg. No. 25,426; Kenneth J. Burchfiel, Reg. No. 31,333; Gordon Kit, Reg. No. 30,764; Susan J. Mack, Reg. No. 30,951; Frank L. Bernstein, Reg. No. 31,484; Mark Boland, Reg. No. 32,197; William H. Mandir, Reg. No. 32,156; Scott M. Daniels, Reg. No. 32,562, Brian W. Hannon, Reg. No. 32,778, Abraham J. Rosner, Reg. No. 33,276; Bruce E. Kramer, Reg. No. 33,725; Paul F. Neils, Reg. No. 33,102, Brett S. Sylvester, Reg. No. 32,765 and Robert M. Masters, Reg. No. 35,603; my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and request that all correspondence about the application be addressed to SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC, 2100 Pennsylvania Avenue, N.W., Washington, D.C. 20037.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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First Name Last Name

Residence _____ Signature _____

_____ Post Office Address _____

Citizenship _____ _____

Date _____ Eighth Inventor _____

First Name Last Name

Residence _____ Signature _____

_____ Post Office Address _____

Citizenship _____ _____

(Supply similar information for ninth and subsequent joint inventors.)